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THE DEVELOPMENT OF PROGRESSION AND POSTURE IN YOUNG OPOSSUMS

ORTHELLO R. LANGWORTHY

From the Department of Anatomy, The Johns Hopkins Medical School

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For the last two seasons it has been possible to study in this laboratory the development of reflexes in pouch-young opossums. The maturing of electrically excitable areas in the cerebral cortex and the mode of onset of rigidity in decerebrated animals have already been reported (Weed and Langworthy, 1925a, 1925b). The present additional observations, increasing the series of pouch-young to 35, renders now possible the further discussion of the development of progressive and postural activities characteristic of this species, correlated with the course of general body development.

The choice of the opossum as an experimental animal for this particular work seems happy since the young are born after a very short gestation-period, when approximately 11 mm. in length. Though the fore-legs are fairly well developed, the hind-legs are merely extensive buds. These vigorous, air-breathing embryos and fetuses may be detached from the nipple in the pouch of the mother for observation and study.

The act of walking may be considered as composed of two definite factors—one, dynamic; the other, static. The former is made up of alternate orderly flexion and extension of the legs inducing progression: the latter is the postural reaction counteracting the force of gravity and enabling the legs to support the body weight.

Sherrington demonstrated in 1896 that this postural reflex could be accentuated by transecting the brain through the mesencephalon; the antigravity muscles then show a continuous and prolonged contraction. The phenomenon brought forth by this operative procedure suggested many experiments of great interest and importance and reports of results of decerebration of the common laboratory animals became soon available. But the complicated mechanics of the reaction and the tracts involved are

still not clearly understood. In another paper these divergences of morphologic interpretation are discussed (Langworthy, 1925).

Further understanding of the postural reflex has been obtained by attacking the problem from the developmental viewpoint. New-born kittens, after decerebration, did not exhibit the extension seen in adults of the series but, on the contrary, became enormously active (Weed, 1917). If suspended in a warm box, they continued alternate progressive movements of all four legs for considerable periods of time. If placed upon the floor they sprawled along at a rate slightly faster than that of normal animals of the same age. The postural factor had not yet developed while the tendency toward progression seemed accelerated by the operation. Rigidity gradually developed in the older preparations as evidenced first by extension and adduction of the fore-legs when at rest, later by a slight resistance to passive movement of the extremities. By the fourth week of life, the postural reactions approached those of the adult. As rigidity came to be dominant, the progressive tendencies were inhibited; during the transition period, the animals tended to show the postural reaction at the beginning of the experiment and to be more active later.

Continuing this work on other species, it was shown that the reactions of new-born animals after decerebration depend to a great extent upon their maturity at birth (Langworthy, 1924). Thus rabbits, born very immature after a short gestation period were very active and the postural factor did not become dominant for some weeks. Guinea pigs, on the other hand, born with the eyes open, possessing a well-developed coat of fur and able to run about almost as actively as adults, never exhibited these active progressive movements after decerebration but all developed rigidity. The gait of the decerebrated young in these species corresponded with the normal mode of progression of the adult. Thus the kittens usually displayed alternate progressive movements, trotting, seldom galloping; the rabbits on the other hand hopped and seldom trotted.

The development of electrically excitable areas in the cerebral cortex of young kittens has been studied (Weed and Langworthy, 1925c). It was found that an area which on stimulation produced flexion of the contralateral fore-leg, was present at birth, and that centers for the facial and masticatory muscles were developed during the first month. Moreover, in the course of development these centers became more delicately differentiated and organized. Olmsted and Logan (1925) found that after removal of definite areas of the cortex in the adult cat, the postural reflex was accentuated. Further developmental observations upon the cortex approached from these viewpoints may add to our knowledge of posture and progression.

These procedures which furnish so much information in mammals have scarcely been applied to lower forms. Rogers (1922), in his studies of the

behavior of pigeons after removal of portions of the brain, gave little information concerning changes in walking.

An opportunity presented itself to extend the experiments to the reptilia. Bagley and Richter (1924) had reported in the cerebral cortex of an alligator the presence of an electrically excitable area, the reactions from which tended to be of the mass variety involving the contralateral legs and tail rather than a single extremity. Later the brain of the alligator was transected at various levels and the effect upon walking studied (Bagley and Langworthy, 1925). It was observed that removal of the entire forebrain was without any apparent effect, but if the entire midbrain was also removed, changes in locomotion occurred. With the first few steps the legs became more and more extended, till the extension in the fore-legs was maximal. This position seemed to inhibit further progression and the animal stood quiet like a statue, high upon its legs. Now, upon strong stimulation, the animal attempted to walk, but lost its balance with the first step so that it fell to the side. When at rest the legs were flexed in normal manner. No resistance to passive movements of the extremities was ever noted. In the alligator, then, after removal of the midbrain the postural reflex apparently became accentuated upon any attempt at progression.

Unpublished observations showed that a similar mechanism can be demonstrated in the pigeon. If the midbrain be removed together with the optic lobes, the legs are normally held flexed close to the body. But if now the claws are spread out on the table and an attempt made to balance the body weight upon the extremities, the legs arrive by a series of jerks at a maximally extended position, so strong indeed that it is impossible for the bird to stand and it falls backward. The extension is lost as soon as the animal falls. Under such conditions no true rigidity has ever been seen.

The experiments on young opossums assume greater significance when interpreted with a broad comparative background, for this animal in its development exhibits the simpler as well as the more complex postural reflexes. In this paper the development of coördinated movements of the extremities will be presented. These coördinated reflexes are already present in the kitten and rabbit at birth. Indeed, the first observation upon young decerebrated animals (Graham Brown, 1915) dealt with one litter of fetal kittens, all of which showed coördinated alternate progressive movements without rigidity.

Formerly, it was believed that opossums at birth were placed in the pouch by the mother. Hartman watched the new-born animals grasp the hair with the claws of the fore-legs and pull themselves into the pouch. The fore-legs themselves are functional and coördinated for grasping and climbing at birth. The hind legs are of course of no service in these movements.

METHOD OF EXPERIMENTATION. The opossums used in these experiments were all obtained from Texas, through the kindness of Dr. Carl Hartman. Some of the litters were born before arrival but in every case it was possible to estimate the age accurately.

Observations were first made upon the general reflex behavior of the young opossums after removal from the pouch. In order, however, to study more favorably the development of crossed reflexes in the cord, the animals were given ether by cone, the sciatic and nerves of the brachial plexus exposed and stimulated.

The animals were later decerebrated, according to a technique described in a previous paper, and the subsequent activity of the animal compared with that exhibited before operation. Nine decerebrated adults were also used as controls. The series of pouch-young now has reached 35, the individuals varying in age from 10 to 89 days. The brain and spinal cord in every case were preserved for morphological study.

EXPERIMENTAL OBSERVATIONS. In presenting the data upon which this paper is based, the activity of characteristic preparations will be discussed, beginning with the youngest group and then including those of older age.

Pouch-young of 10 to 50 days. The first observations in the series (PY1) deal with the activity of 8 animals measuring 22 mm. crown-rump and 27 mm. nose-rump, approximately 10 days of age. These pouch-young opossums had the bodily characteristics of fetal animals, with large heads, fairly well developed fore-legs and poorly developed hind. No coat of fur had appeared; the skin was pink, greatly wrinkled and almost transparent, exposing clearly to view the sutures of the skull, the liver and other abdominal organs. A round opening served for a mouth, traumatized by freeing from the nipple, and no mouth groove had yet appeared. A ring of pigment in the iris indicated the position of the developing eye. The pinna of the ear had not separated from the skin. The fore-legs were muscular and had well developed claws, the hind-legs were one-third their size and poorly differentiated. No pouch or scrotal sac could be seen so that it was difficult to determine sex.

The animals on removal from the pouch were very active and squirmed constantly. The general body musculature contracted well, flexing the back and arching the body to the side. The predominant reaction to all stimuli was this constant general contraction. The opossums could not stand well or support their weight upon their legs nor was there any tendency toward progression when put upon a smooth surface. If placed upon gauze, however, the threads were seized with the claws of the fore-legs and the animals pulled themselves along. They could indeed support the weight of the body by one fore-leg hanging to the gauze. The tail at this time was becoming prehensile but could not yet support the weight of the body.

When the animals lay on their side or back, the fore-legs flexed and extended in well coördinated alternating rhythm. The hind legs, on the other hand, beat only occasionally, poorly coördinated in time with each other and not at all with the fore-legs. The whole posterior portion of the body was moved together by mass contraction of the ventral body musculature. The activity was indeed continuous and futile in character. If a unipolar electrode was placed on the plantar surface of the fore-foot the claws immediately closed around it. Stimulation anywhere simply accelerated the movements already described.

Operative procedures added little to the information already obtained. Decerebrated specimens acted in all respects like normal animals, showing the same press of spontaneous activity, although to less degree. When placed in water these very young unoperated opossums could swim, although they were not able to keep the head out of the water.

The reaction of a second group of seven animals may be considered together (PY2, PY3, PY4, PE18, PY5, PY6, PE34). They varied in age when studied from 14 to 41 days, in crown-rump measurement from 27 to 56 mm. and in nose-rump from 40 to 64 mm. In the youngest, the mouth slit could first be distinguished; in those over 18 days old the eye slit was forming. In all, the development of the sinus hairs could be noted. The pinnae had separated from the underlying skin. A pouch or scrotal sac could be distinguished, aiding in the determination of sex, while the testes were as yet undescended. In the older a coat of down was appearing upon the body. The fore-legs in all were much more strongly developed than the hind.

The voluntary movements of these animals when detached from the mother differed very little from those already described. The opossums grasped the gauze with the fore-legs and so pulled themselves along. They could not stand, and if placed in an upright position fell immediately to the side. Movements of the hind-legs were few and almost completely incoördinated. But there were indications of increased motor coördination. Thus the two oldest could support their weight hanging by the tail. Their ability to climb was tested. The four oldest clung to a rough rope with the fore-legs but showed no tendency to climb, rather curling the body around the rope and soon falling. The two oldest made the rasping noises so characteristic of older opossums.

It was particularly desired to test in this group the development of crossed reflexes to the cord. As the normal animals were so active that results of electrical stimulation of the body surface could not be interpreted, ether was given and the sciatic and brachial nerves were exposed for stimulation. While the results were not as clear-cut as might possibly be wished, it was very evident that crossed reflexes were much more easily obtained in the fore-legs than in the hind-legs. A relatively strong current

was necessary, even for the stimulation of the nerves of the brachial plexus, and excitation of the sciatic would cause movements of the fore-legs almost as soon as of the opposite hind. In order to obtain any response at all it was imperative that the anesthesia be very light. The peripheral nerves at this time showed grossly no trace of a myelin sheath.

The activity of the decerebrated specimens in this group was almost that of intact preparations. The length of the period of etherization necessary for the other procedures tended to cut down the subsequent progressive activity.

The next eight animals (PY7, PE38, PY8, PE42, PE45, PE51, PY9, PY10) were from 41 to 49 days of age and varied in crown-rump length from 56 to 71 mm.; in nose-rump length from 64 to 75 mm. Their development in tangible factors was relatively slow. The mouth slit slowly differentiated and in the oldest was opening at the corners. The eye-slits and pinnae continued to develop. The fur grew slowly and even in the oldest was not yet conspicuous. The skin, however, was less creased and transparent; and the sutures were no longer plainly visible. The vibrissae were long, the pouch or scrotal sac well formed. The hind-legs were still considerably less developed than the fore.

The older of this group were able to stand after a fashion, the fore-legs offering good support, the hind sprawling laterally. The neck muscles had become strong so that the head was effectively supported; the tail was curved under the body and used for balancing. But on any attempt at walking, the opossum fell to the side. Movements of the hind-legs were still few and uncoördinated. All eight could hang by the tail. The oldest climbed the rope poorly, but as the eyes were not yet open, the movements were over done and far from accurate. Crossed reflexes, obtained on stimulation of isolated nerve trunks, were still more easily obtained and more active between the fore-legs than between the hind.

The opossums after decerebration were not exceedingly active. A few beats of the fore-legs were obtained, ceasing almost as soon as the stimulus was removed. The hind-legs participated to about the same extent as in the intact animal. In one preparation, the thoracic spinal cord was exposed and transected at the end of the experiment by a quick cut of the scissors. The hind-legs were immediately drawn up into flexion and remained in this position. The fore-legs were stimulated to alternate progressive movements continuing a brief time.

Pouch-young of 51 to 89 days. The next largest opossum (PE54) was 74 mm. in crown-rump measurement and 56 days old. The animal stood and walked fairly well, showing a great tendency to progress backward and making continuous sucking noises. After stimulation of the cortex the opossum was decerebrated.

For the first time in this series, great activity was exhibited by this animal

after transection of the brain. Ten minutes after coming out of ether, this opossum became active. When tickled on the pinna or at the base of the ear a scratch reflex was obtained. It would grasp objects with its tail but the flexion of the tail could no longer support the animal's weight.

In less than an hour, prolonged progressive movements began, there being, however, poor coördination between the legs, the fore-legs beating at a faster and more perfect rhythm than the hind. The animal stood and walked almost as well as before the operation. There was no evidence of any extreme extension or rigidity during the experiment. The weight was poorly sustained by the hind-legs which tended to sprawl to the side. The walking was continuous for thirty-seven minutes. There ensued upon transection of the spinal cord in the thoracic region, extreme flexion of the legs followed by a few alternate beats ending in extension. The tail became extremely flexed, curled like a coiled rope.

Four animals (PE62, PE70, PE71 and PY11) varied from 62 to 68 days in age and in measurement, crown-rump from 82 to 110 mm., nose-rump from 92 to 120 mm. They differed from all opossums previously considered in that their eyes were open. They were not yet miniatures of adults, the head and particularly the pinnae were large proportionately. The hind-legs were still much smaller than the fore. They continually made loud sucking noises, hung with ease by the tail and climbed a rope with facility. In standing, the hind-legs did not yet offer adequate support to the body. They walked, however, fairly well, showing almost a predilection to progressing backward. They responded extremely actively to auditory excitations. Stimulation of the sciatic nerve after decerebration in one of the opossums gave very little evidence of crossed reflexes in the opposite hind-leg.

After decerebration, all four showed accentuated progressive activity, similar to that already described for kittens and rabbits. This consisted in constant alternate flexion and extension of the legs, there being little coördination, so that the fore-legs beat more rapidly and continuously than the hind. There was no evidence of any extreme extension or rigidity in the legs. The preparations could apparently stand about as well as before the transection. Moreover, they could walk and one did walk continuously for seventeen minutes, tending to be a little ataxic on its feet.

The ease with which the scratch reflex could be elicited in these decerebrated animals by stroking the ear was remarkable. The animals also gave the characteristic cry and responded actively to loud noises. Cutting the spinal cord in the thoracic region was followed by a few alternate beats of the hind-legs ending in extension.

Opossum PY12, with crown-rump measurement of 108 mm., was only slightly older than the animals first considered. About three minutes after decerebration, stimulation of the footpads in this animal started the

progressive movements which continued uninterruptedly for an hour and three-quarters. At first, these movements were almost entirely of the fore-legs which beat at a rather rapid rate. The left hind-leg participated at infrequent intervals, the right hind-leg remaining quiet and extended. This progressive tendency was first observed with the opossum lying upon its back, and when turned over it balanced its weight upon its feet and walked. The legs after the first few steps became more and more extended till the preparation stood with extreme extension of the fore-legs and more extension of the hind than was present normally. At the same time, maximal extension of the neck and some dorsal bowing of the back was apparent. In walking, the animal raised the fore-legs high like a thoroughbred horse. Perfect alternate movements of the fore- and hind-legs were seen at times but more often the rhythm of the fore-legs was much faster than that of the hind. The hind-legs and tail were used in balancing. Indeed, near the close of the observations when periods of rest intervened, the opossum crouched upon his hind-quarters in order to maintain equilibrium.

During the early periods of the experiment the animal was so spontaneously active that it was impossible to inhibit these movements. For the most part, it walked in small circles to the right. The opossum not only made the ordinary crying noises but often yawned. It responded readily to noises. Its ability to climb a rope was tested. It grasped the rope with the forelegs but could not climb because the greater extension of the neck and back caused it to fall backward. Stimulation of the body surface gave very little evidence of crossed reflexes in the hind-legs. After quickly cutting the thoracic cord the tail became extremely flexed and coiled, then slowly uncurled, to come finally to rest in extreme extension.

Opossum PY13, 118 mm. in crown-rump and 135 mm. in nose-rump measurement, was the youngest animal to stand steadily on its feet, simulating the adult. But although walking was active, more dependence was still placed upon the fore-legs than on the hind. The discrepancy in size between the fore- and hind-legs had largely disappeared and progression was clearly of the alternating type.

Immediately after decerebration, progressive activity of the fore- and hind-legs followed the alternative type very accurately. After an extremely short time, however, the movements of the hind-legs grew slower and finally ceased, leaving the fore-legs beating. When placed upon its feet, the legs were so extended that balance was impossible and the animal fell. No long periods of progression occurred and no rigidity was noted in the legs. The opossum, when undisturbed, lay quiet with extremities flexed and flaccid; when stimulated a few progressive movements were obtained on maximally extended legs with head, neck and tail also maximally extended.

The scratch reflex was very easily obtained by stimulating the ventral surface of the body from the neck region to the lower edge of the pouch. It was particularly easily elicited around the pouch and could not be obtained by stroking the base of the ear. Again, on cutting the thoracic cord the extreme flexion of the tail occurred, being accompanied by a continuous writhing movement and finally ending in extension.

The six older animals in the series all had a good coat of hair and the teeth were penetrating the gums. The younger (PY14, crown-rump 110 mm., nose-rump 125 mm.) was 76 days old. It ran very fast and often walked backward. Loud noises greatly startled it. After decerebration, the scratch reflex was at first the dominant reaction, elicitable from all parts of the ventral surface of the body. The animal attempted to walk but the maximal extension of the legs prevented more than a few steps and caused the opossum to fall to the side. At times, extension and transient rigidity were observed in the quiescent animal. The scratch reflex remained extremely active and on carrying the point of stimulation across the midline the same leg continued to beat. When the thoracic cord was cut the tail writhed into extreme flexion, later becoming extended; at the same time a few beats of the hind-legs occurred, ending in extension. The fore-legs also showed rhythmic beats, finally becoming extended and rigid.

The next oldest animal (PY16, 80 days old, 136 mm. crown-rump measurement) exhibited after decerebration the same extension upon any attempt at walking, the extension being most marked in the fore-legs and neck, but also prominent in the hind-legs, back and tail. This extensor out-thrust seemed to inhibit after an extremely short time any tendency toward progression. True extensor rigidity in the fore-legs was noted at times.

The three oldest opossums of the series of young animals (PE45, 84 and 93) showed after decerebration the same tendency toward extreme extension when attempts at progression were made by the animal. When quiet the extremities were often flexed. Sometimes, however, weak and transient extensor rigidity was noted, particularly after attempts at walking. The intense resistance to passive motion seen in adult decerebrate animals was, however, not found.

It may appear that the behavior of this group of decerebrated animals differed considerably from that of adult opossums, after similar experimental procedures. But, as has been pointed out in a previous paper, the rigidity in the adult opossum while very similar to that exhibited in the common laboratory mammals is not as constant as in the cat and dog. In the adult opossum rigidity developed strongly in the fore-legs but it took considerable time for it to appear in the hind. Moreover, the rigidity was interrupted, without any apparent cause, by periods of passive flexion or by rhythmic beats of all four legs.

Discussion. Sherrington (1910), Forbes (1912) and Graham Brown (1914) have in recent years added much to our knowledge of the nature of progression, showing that it is a fundamental activity of the nervous system,—rhythmic in nature and dependent upon a balance between two central, antagonistic forces, one tending toward flexion, the other toward extension. Now one force, now the other, becomes dominant, the antagonistic activity being successively in a state of inhibition. The balance of these activities leads to alternate flexion and extension of the extremities.

Moreover, while normally controlled by higher centers, this tendency to rhythmic activity, as shown by the experiments of Sherrington, is clearly also a fundamental property of the spinal cord. He obtained evidence which indicated that the proprioceptive fibers from the muscles formed the afferent link in the reflex arc for the leg. Graham Brown (1912) afterward demonstrated, however, that the integrity of this proprioceptive pathway was not essential. He exposed and quickly cut the thoracic portion of the spinal-cord in animals whose hind-legs had been completely deafferented and observed several rhythmic beats of the hind-legs which finally came to rest in a position of extreme extension. The rhythmic tendency in this experiment at least, seemed to be maintained on the motor side of the reflex arc. Brown considered that under certain conditions such as asphyxia, these motor centers might be influenced to rhythmic activity by substances contained in the blood stream after the manner of the respiratory center.

In further experiments to test this theory of rhythmic coördination on the motor side without the intervention of the afferent portion of the reflex arc he showed that rhythmic movements of the extremities occurred in cats at a depth of narcosis at which the afferent pathways were no longer excitable (Graham Brown, 1914).

Laughton (1924) found that in the adult cat and dog two-thirds of the thalamus and in the rabbit the cephalic two-thirds of the pontine region must be intact in order that spontaneous progressive movements might occur. But Weed (1917) had already clearly demonstrated that in newborn kittens, after decerebration, the tendency to coördinated rhythmic beats of the legs is accentuated.

Similarly, experiments made by the writer (1924) on decerebrated newborn kittens and rabbits gave evidence that the progressive tendency was already developed and coördinated. In such animals the fore- and hind-legs tended to beat in regular alternate rhythm. The postural reflex, however, was as yet not manifested, either as an extension of the legs or resistance to passive movement of the joints. Animals of greater maturity at birth (guinea pigs) exhibited after decerebration the typical extensor out-thrust of decerebrate rigidity, without progressive rhythms.

The opossum at birth does not show, either in the normal state or after

decerebration, coördinated movements of all four extremities. The front legs, indeed, are well developed and are flexed and extended in alternate rhythm. The hind-legs, on the other hand, are seldom moved and then are not well coördinated with each other or with the fore-legs. This lack of coördination is even more clearly brought out when the sciatic nerve is stimulated under anesthesia. Movements of the fore-legs were elicited upon stimulation of this nerve almost as easily as movements of the opposite hind-leg. The central nervous connections of the hind-legs in such animals are apparently not yet fully functional. Indeed, perfect coördination between all four extremities of the opossum develops relatively late in pouch life. The precocious development of the fore-legs may be partially in response to a definite need. We have, however, in all mammals proof of the earlier development of the fore-leg embryologically.

The appearance of the postural reflex in the opossum may now be discussed and the manner in which it comes to dominate the picture in the decerebrate preparation. The younger animals, when decerebrated, showed in general the same reactions as the intact animal although they were less active than in the normal state. At about the time that the eyes opened the general activity of the animals after such transections changed. The tendency to progression became greatly accentuated so that the animals walked constantly, even though deprived of the upper portion of the central nervous system. No tendency toward extension of the legs was apparent at this stage. This activity corresponded to that of the new-born kittens and of rabbits, to which reference has already been made. The progressive tendency had become quite perfected and active and at this stage dominated the picture. The hind-legs of the opossum beat irregularly at a slow rate, uncoördinated with the fore-legs, while the alternate rhythm of all four legs of the kittens and rabbits was more perfect.

Older decerebrated opossums exhibited the first appearance of a postural reflex and although extremely active, walked with extremities maximally extended. This phenomenon in the decerebrated opossum may be compared with the activity of adult alligators whose pallium and midbrain had been removed (Bagley and Langworthy, 1925). This hyper-extension of the legs in both the opossum and alligator was not constant, but appeared only upon attempts at walking. The development of this posture in the alligator inhibited further progressive movements and the animal stood quiet on extended legs. If maximal stimulation was applied so that the alligator was incited to walk, balance was no longer maintained. In slightly older opossums, indeed, the great extension made walking movements impossible and after falling to the side the animals would lie quiet. The development of the postural reflex seemed, therefore, to inhibit the progressive tendency. In still older decerebrated preparations the appear-

ance of rigidity in the extended legs completed the picture of the adult preparation. But in the adult decerebrate opossum, in contradistinction to the cat and dog, periods of rhythmic progression tended at times to break in upon the extensor rigidity.

Camis (1923) discusses in a recent paper the central coördination of posture and progression. In relation to the double function of the muscle to maintain posture and to produce progression, there may be found in the brain two centers whose stimulation produces respectively tonic reflexes and rhythmic reflexes. As progression is produced by the balancing of antagonistic centers, so these may be antagonistic, one tonic and the other phasic; when one is dominant the other is inhibited.

At a certain period in the development of the opossum, before the postural reflex appears, the progressive tendency, already developed, is accentuated after decerebration. In slightly older animals, which after decerebration, walk constantly on extended legs, the two mechanisms seem to be fairly evenly balanced. With the increasing development of posture, progression tends to be inhibited by the postural mechanism which comes to dominate the picture. But even in adult opossums this tendency to rhythmic activity tends to break through. In adult decerebrate cats a very strong stimulus is required to produce flexion of an extremity.

The appearance of extensor rigidity in adult decerebrate animals is apparently due to a release from the normal controlling influence of higher centers. Weed (1914) found that stimulation of the medial third of the cerebral crura inhibited the rigidity; tracing this pathway cerebralwards he demonstrated that these inhibitory fibers formed a part of the fronto-pontine tract. Camis (1923) has confirmed the observation of Weed that the cerebellum forms a link in this inhibitory pathway.

Warner and Olmsted (1923) searching for the cortical representation of this function, found that if the frontal lobes were removed in the cat, extension of the extremities resulted. If the motor cortices were left intact, this extension could be overcome and walking occurred. Stimulation of the surface of the wound inhibited the extension. A more recent communication (Olmsted and Logan, 1925) adds but little to our knowledge of this phenomenon as the operation areas are poorly defined. Moreover it is difficult in these chronic experiments to gauge accurately the actual extent of the injury.

SUMMARY

A series of pouch-young opossums were subjected to decerebration, with transection of the brain stem at the level of the superior colliculi. The reactions of these animals were compared with those exhibited in the normal state, cutaneous reflexes, posture and progression being especially

noted. The reactions observed permit a grouping of the animals according to age.

In the first group (pouch-young opossums of less than 50 days of age) the same types of reflex and progressive activities were exhibited after decerebration as in the normal state, but these activities were of lesser degree. Animals of slightly older age (about 56 days), showed after decerebration outspoken tendencies to prolonged, unceasing progression of all four legs, more marked than before the transection. And in a still older group (pouch-young of about 64 days), prolonged progression on maximally extended legs characterized the decerebrate state. In the oldest group of pouch-young opossums (about 84 days) the extensor out-thrust of true decerebrate rigidity appeared after the transection of the neuraxis, the progressive tendency being thereby suppressed.

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A CONDITIONED SALIVARY REFLEX ESTABLISHED BY CHRONIC MORPHINE POISONING

K. H. COLLINS AND A. L. TATUM

From the Laboratory of Pharmacology of the University of Chicago

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Conditioned reflexes have been observed from time to time under various circumstances. Cason (1922) produced a conditioned pupillary reaction in man by the ringing of a bell and at the same time subjecting the eye to stimulation by a strong light. After daily repetitions for a time, ringing the bell alone produced the pupillary reaction. A conditioned lid reflex was produced in a similar manner. Pavlov's classical experiments in psychic salivary secretion (1910) are well known. Dogs with salivary fistulae were fed simultaneously with the ringing of a bell, and the salivary secretion collected from the fistula. After this had been continued for some time ringing the bell alone was sufficient to excite the secretion of the saliva.

It is the object of this paper to report, without further analysis, a conditioned salivary reflex primarily initiated by morphine. This was observed during the course of some experiments in chronic morphinism.

Dogs were given subcutaneous administrations of morphine sulphate daily. A small amount of morphine (30 to 80 mgm. per animal) produces in the dog salivation and usually emesis and defecation. After seven or eight daily administrations of morphine these dogs salivated profusely and even in a few instances vomited *before* the morphine had been given. The entrance into the dog room of the person conducting the experiment was in most cases sufficient to precipitate this copious salivation. The sight of a hypodermic syringe never failed to produce a secretion of saliva, which continued for a variable length of time, occasionally for several hours providing distractions, such as feeding, did not occur, or the reaction stopped by the narcosis produced by the subsequent administration of morphine.

If this salivation is secondary to nausea, it might be suspected that in this condition the ability to swallow was impaired. That this cannot be the case is evidenced by the fact that the dogs were observed to drink and on a few occasions to eat during this period of salivation.

Pain cannot be a factor since morphine itself is the most powerful of analgesic agents. Furthermore, fear was never at any time evident in these animals.

Eight dogs have been observed in this study of chronic morphinism and all have developed this phenomenon after seven or eight days. One cat has developed a similar condition after fourteen days, differing from the dog only in the ease with which the reflex is established. Three of these dogs have been receiving morphine daily for a period of four months and at the time of the present writing still salivate at the sight of a hypodermic syringe.

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STUDIES ON THE EFFECT OF ABDOMINAL PRESSURE ON URINE VOLUME

JOHN Q. GRIFFITH AND HOWARD R. HANSELL

From the University of Pennsylvania, Philadelphia

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H. C. Bazett and J. B. S. Haldane (1921) demonstrated the occurrence of a diuresis in baths. Later, a more detailed series of studies was reported by Bazett and his associates (1924a and b). In these, an attempt was made to distinguish between the effects of the temperature and those of the bath proper. It was decided that various circulatory and respiratory changes observed were dependent mainly on changes in skin and body temperature, as analogous results were obtained in hot rooms. The diuresis was obtained only in the baths, and seemed relatively independent of the temperature of the bath. In all these experiments, it was found that the character of the urine was influenced very largely by those factors which cause normal diurnal variations.

The diuresis in the baths appeared to be dependent on the water pressure over the abdomen and not on variations in temperature, and consequently we designed our experiments to determine whether abdominal pressure maintained by any other method could also give a diuresis.

Methods employed: Diurnal variations in urine secretion have been conspicuous in our experiments, and therefore, in order to exclude them as much as possible, comparison has been made between the rate of urine secretion on control afternoons and that on others when pressure was maintained on the abdominal wall. The routine adopted was as follows: on the morning of the experiment the subject ate breakfast as usual, but from then until the close of the experiment neither food nor drink was taken. Urine was passed at 9 a.m. From 9 to 1 the subject engaged in ordinary classroom and laboratory routine. In general, urine was collected at 12 and again at 1 p.m. From 1 to 2 the subject lay down, and urine was collected again at 2 p.m. Then pressure was applied, from 2 to 3 and from 3 to 4, and urine was collected at 3 and 4 p.m.

An even pressure was distributed over the trunk by means of a large rectangular rubber bag held in place by cloth bands which were pinned in place at the back, so that the bag covered the anterior surface of the trunk reaching from the arm pits to the hips between the anterior axillary lines. Air was pumped in by means of a rubber bulb attached to one arm of a T

valve, the other arm of which was connected to a water manometer. The pressure used was either 10 or 25 cm. of water.

The temperature of room was always high. Only the outer clothing was discarded. At no time in any of the experiments was there any feeling of cold.

Control experiments were run on other days in exactly the same way except that no pressure was applied. In some of the controls the bag was put in place and in others not, but this factor did not appear to make any difference.

Urine was collected under paraffin, but was passed through air. The pH was determined by the colorimetric method; total acidity by the neutral oxalate titration method; ammonia sometimes by aeration, and sometimes by Malfatti's formaldehyde method and sometimes by both; urea by the Van Slyke aeration method; chlorides by the Volhard method.

TABLE 1

CONDITIONS	SUBJECT	AVERAGE VOLUME IN CUBIC CENTIMETER MOVING ABOUT	AVERAGE VOLUME IN CUBIC CENTIMETER FIRST HOUR LYING DOWN	AVERAGE VOLUME IN CUBIC CENTIMETER SECOND HOUR LYING DOWN	AVERAGE VOLUME IN CUBIC CENTIMETER THIRD HOUR LYING DOWN	PERCENT INCREASE	NUMBER OF EXPERIMENTS
Control	J. Q. G.	69	69	76	86	17	4
10 cm. pressure	J. Q. G.	84	62	149	162	150	4
25 cm. pressure	J. Q. G.	86	106	101	78	−15	3
Control	H. R. H.	42	40	52	52	30	3
10 cm. pressure	H. R. H.	47	47	54	74	36	2
25 cm. pressure	H. R. H.	69	67	100	161	95	3
Pressure somewhat over 15 cm	H. R. H.	93	72	182	250	200	1

Results obtained: In some of our experiments we were able to measure the volume of the urine excreted during the last hour moving about, but often this was impossible and the urine had to be taken in one composite three- or even four-hour sample. Obviously the average hourly output over this longer period would be somewhat high as compared to the output in the last hour, for in the early hours of the morning there would be the diuresis following breakfast. Therefore, using such average figures, lying down might not show a diuresis even if one existed, which is not particularly important as comparisons have not been made to the morning rate of excretion. In actual practice the excretion of urine during the first hour lying down was slightly less than the average amount per hour for the previous three hours moving about (67 cc. as compared to 69 cc.), though in view of the above mentioned considerations this may represent in reality a slight diuresis. In the second and third hours lying down a definite

diuresis is usually seen even on the control days. In table 1 this diuresis is expressed as the average percentage increase above the excretion in the first hour lying down. Pressure was applied on the experimental days in

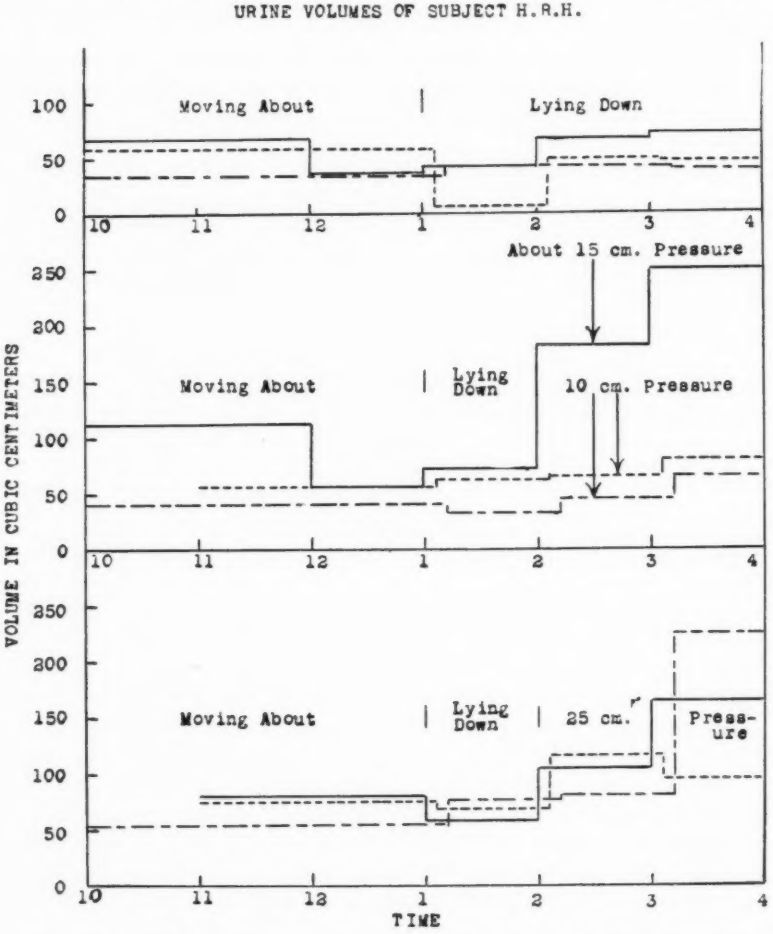

Fig. 1. Actual figures of all experiments on H. R. H. Upper 3 curves control day. Middle curves—2 with 10 cm. water pressure and one about 15 cm. Lower 3 curves with 25 cm. pressure.

the second and third hours after the lying down period, and in table 1 the average increase in the urine excreted per hour for these two hours is compared with that of the first hour, showing a much greater increase than is seen on the control days.

It is seen that subject J. Q. G. got a moderate but well sustained diuresis on simply lying down. On application of 10 cm. water pressure he obtained in every case a marked diuresis, averaging a 150 per cent increase. But with 25 cm. pressure he never got a diuresis, and the hourly volume fell below that of the first hour lying down. Subject H. R. H. on the other hand, never obtained more than a slight diuresis with 10 cm. pressure, but with 25 cm. pressure the hourly volume increased more than 90 per cent. The experiment starred is one in which the pressure is not accurately known. It was the first experiment of the series run with pressure, in the nature of a preliminary trial, and the pressure was kept high, certainly above 15 cm.

The results would seem to indicate that there is an optimum pressure for producing diuresis. Pressure too far above the optimum may fail to cause a diuresis, and even produce suppression. Thus it would seem that

TABLE 2

TIME	CONDITIONS	VOLUME	pH	TITRATABLE ACID IN MILLI EQUIVALENTS	NH_3 IN MILLI EQUIVALENTS	UREA IN MILLIMOLS	CHLORIDE IN MILLI EQUIVALENTS
			Subject J. Q. G. (control)				
		cc. per hour					
9–1	Moving about	90	6.8	0.66	3.6	8.3	8.4
1–2	Lying down	51	5.6	0.40	1.22	14.4	7.67
2–3	Lying down	66	5.5	2.12	0.92	12.5	11.3
3–4	Lying down	78	5.9	1.8	0.94	16.8	13.4
			Subject J. Q. G				
9–12	Moving about	113	6.6	1.1	1.0	19.6	12.7
12–1	Moving about	107	6.2	1.7	1.7	15.9	10.7
1–2	Lying down	21	5.8	3.4	3.2	2.1	
2–3	10 cm. pressure	237	5.8	4.74	2.4	21.5	21.7
3–4	10 cm. pressure	180	6.2	2.8	1.6	14.0	17.2

10 cm. was near the optimum pressure for subject J. Q. G., while for H. R. H. 25 cm. was probably nearer his optimum than 10. The starred experiment might suggest that his optimum was really above 15 cm. All the results of subject H. R. H. are plotted in figure 1.

The character of the urine obtained is shown in table 2, in which two experiments on subject J. Q. G., one a control, are listed. Acidity and pH show their usual diurnal variations, the urine being more alkaline in the morning than in the afternoon. Using phenol phthalein as an indicator in titrating for acidity, the curve of ammonia elimination parallels that of acid excretion. Chlorides, at the time of the diuresis, decrease in concentration, but the total output is increased. In two experiments with marked

diuresis in which ureas were determined, the urea excretion increased slightly, but not nearly in proportion to the increased volume.

DISCUSSION: The figures reported indicate quite definitely that abdominal pressure can produce a diuresis and that for any subject there is probably an optimum value, so that pressure above this may cause no increase or actually a decrease in volume. The pressures we have employed are low, about comparable to the hydrostatic pressure on the abdomen in the baths, but much less than those used in animal experiments by Thorington and Schmidt in which they investigated the effect of abdominal pressure on urine formation. With the pressures that they used it seems certain that we also would have obtained a diminution in urine volume, so there is really no conflict between their results and ours. They also notice no diminution in urine volume and occasional increases with lower pressures. There can therefore be little doubt that pressure is the dominant factor in the bath diuresis.

The actual mechanism by which these results are brought about is not clear and considerable experimentation preferably in animals would be required to elucidate it. As it is impossible for us at the present time to undertake such experiments, the above results are presented so that this factor may receive more quantitative attention.

As far as deductions are allowable from a small number of data, our findings do not indicate any origin of the fluid from the intestinal contents, if a raised urea excretion is to be taken as evidence of such an origin, as was done by Bazett and his associates. In every other way the type of urine secreted has been similar to that obtained in the bath experiments, but we have never observed a high rate of urine secretion with a maintained high urea percentage. Whether this difference depends on the fasting condition of the subject or on individual differences or on some other factor is not clear.

It would seem inevitable that the venous pressure in the abdomen would be raised as the result of the external pressure, as was demonstrated in the experiments of Thorington and Schmidt (1923). Richards and Plant (1922) showed that a slight increase in the pressure in the renal vein might cause a diuresis in experimental animals, and consequently some such factor is more probably the cause of this condition.

CONCLUSIONS

1. A diuresis analogous to that obtained in baths, occurs upon the application of appropriate pressure to the abdomen.

2. The evidence would seem to indicate that there is an optimum pressure for producing diuresis, different for different individuals, and that the more nearly the applied pressure approaches the optimum pressure the

greater the diuresis. Thus too low pressures might be ineffective, while too high pressures may even cause partial suppression.

3. It would appear that the cause of the diuresis on application of pressure should be sought in connection with increased venous pressure or other physical phenomena, rather than in greater urea excretion.

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GLYCONEOGENESIS

J. MARKOWITZ

From the Physiological Laboratory, University of Toronto, Canada

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When Claude Bernard discovered glycogen, he regarded it as part of the internal secreting mechanism of the liver. Successive physiologists looked upon glycogen more as a storage material, analogous to starch in plants. But if glycogen were merely a storage material, it should be possible to exhaust it by starvation. As a rule this is not so; in fact it may accumulate during starvation. In the case of a dog that had starved for 28 days, Pflüger found that the liver contained 4.8 per cent (22.4 grams), and the muscles 0.16 per cent (20.7 grams) glycogen. Even in pancreatic diabetes the tissues do not become glycogen-free, this substance actually increasing in the heart. Removal of the liver of a depancreatized dog causes as rapid a disappearance of blood sugar as in non-diabetic animals (Pearce and Macleod, 1913). This observation proves that the blood sugar of diabetic, as well as of normal animals is maintained by the liver. As judged by the respiratory exchange, the glycogen reserve of a starved rabbit should be used up in a few hours. That this does not occur indicates that glycogen is being replaced while it is consumed. These and many other observations render necessary a revision of our views regarding the function of glycogen. They suggest that glycogen may serve as an essential intermediate step in the metabolism of carbohydrate, and that the absolute quantity of glycogen in the organism is a balance between glyconeogenesis and glycogenolysis. It is very significant that nearly all the known hyperglycemias are dependent upon the glycogen content of the liver, and even in pancreatic diabetes, the blood sugar practically disappears when the liver is removed.

It was demonstrated by Pollak (1909a) in a series of carefully controlled experiments that repeated injections of epinephrin into glycogen-free rabbits eventually led to a deposition of liver glycogen in quantities commensurate with those found in carbohydrate-fed animals. The rabbits were given about 4 mgm. epinephrin daily for about a week. Reducing sugar in amounts varying from traces to 8.6 grams appeared in the urine during treatment with epinephrin. This observation supports the view that glycogen is no mere storage material, but is a balance between glycogen formation and glycogen destruction. It is confusing, however, in view of

the familiar property of epinephrin in ridding an animal of its glycogen stores (Gatin—Gruzewska, 1906).

The literature on the subject of epinephrin hyperglycemia is contradictory. Much of the work may be criticized on two counts; *a*, Mere starvation may not make an animal glycogen-free. *b*, The absence of glycosuria does not rule out epinephrin hyperglycemia. We have often seen blood sugar values of 0.25 per cent in starved cats and dogs injected with epinephrin, with no accompanying glycosuria. Blum (1901) stated that subcutaneous injection of epinephrin into a dog presumably rendered glycogen-free by starvation resulted in glycosuria. Without giving figures, he stated that there was no significant alteration in the nitrogen excretion of the urine. A year later he retracted his opinion, having observed that continuous starvation of a thin dog resulted in a condition in which injection of epinephrin evoked no glycosuria. His former results he attributed to the fat condition of his animals (1902) an explanation which is significant.

Bang (1913) injected adrenalin subcutaneously into starving rabbits. The resultant hyperglycemia and glycosuria were equally as great as in fed controls. The onset of the hyperglycemia occurred fifteen minutes later in the starving animals.

Michaud (Bang, der Blutzucker, p. 87) injected 8 to 10 mgm. epinephrin into dogs with an Eck fistula, with no change in blood sugar, presumably because the liver in such animals is glycogen-free. If simultaneously the animals were fed sugar, considerable hyperglycemia, up to 0.46 per cent ensued. The use of animals with an Eck fistula vitiates to some extent these observations, as the animals are in a highly unphysiological state.

Drummond and Paton (1904) found that daily repeated injections of large doses of epinephrin into fed rabbits permitted the accumulation of as much liver glycogen as in controls. Ringer (1910) found no increased glycosuria when he injected epinephrin into dogs completely under the influence of phloridzin.

Total removal of the liver in dogs results in a fall in blood sugar accompanied by symptoms of hypoglycemia (Mann and Magath, 1922). Circulating blood sugar is evidently manufactured in the liver, presumably from glycogen. If liver glycogen be an essential product in carbohydrate metabolism it should correspondingly be impossible to induce hyperglycemia, as exemplified by epinephrin hyperglycemia, when this substance is demonstrably absent from the liver. The work of Michaud and of Ringer, cited above, may be objected to on the grounds that their experimental methods were too artificial, so that their conclusions may not be valid in the normal organism. The work of Bang may be criticized on the grounds that his rabbits were not necessarily glycogen-free. In our experience starvation only rarely renders a rabbit glycogen-free; failure to consider

this possibility is the cause of many misinterpretations in the literature of epinephrin hyperglycemia.

Contradictory as all these experiments seem at first sight to be, nevertheless they suggest very strongly that glycogen is not a static, but a dynamic substance, which the organism is always consuming, and almost as quickly replacing. The experiments of Bang (1913) and Pollak (1909) referred to above admit of no other interpretation.

The present work is concerned with a more definite experimental demonstration of these propositions regarding the rôle of glycogen in metabolism.

RESULTS. Our method of rendering rabbits glycogen-free was to starve them in the cold for at least three days. Enough strychnine sulphate was injected subcutaneously to induce vigorous convulsions, 0.45 mgm. per kilo being sufficient. Rabbit 5 attests the efficacy of this procedure.

TABLE 1

Rabbit 5. Starved in cold for 3 days. Temperature was in neighborhood of 0°C. Weight at end of this time was 2800 grams.

2:50 p.m.	1.12 mgm. strychnine sulphate subcutaneously
3:15 p.m.	Rabbit hyperexcitable and tremulous
3:30 p.m.	0.28 mgm. strychnine sulphate subcutaneously
3:45 p.m.	Rabbit developed a state of tetanus with frequent typical convulsions every ten minutes
5:35 p.m.	Rabbit stunned for removal of tissue for glycogen estimation. Liver, heart and skeletal muscle were glycogen-free, i.e., less than 0.01 per cent glycogen (Pflüger's method)

The experiment was performed of injecting epinephrin into such a glycogen-free rabbit, and following the behavior of the blood sugar. Just prior to the experiment a piece of the left lobe of the liver was removed for estimation of glycogen, hemorrhage being avoided by a tape ligature. This was done under anesthesia with "amytal."[1] Epinephrin was injected, and samples of blood were removed at intervals for sugar estimations (Shaffer-Hartmann, 1921).

In rabbit 24, the liver was almost but not quite glycogen-free. Injection of epinephrin evoked no hyperglycemia.

In rabbit 26 six hours elapsed between the strychnine convulsions, and the injection of epinephrin. The liver contained 0.120 per cent glycogen. In 1½ hours the blood sugar rose to 0.194 per cent and post mortem the tissues were glycogen-free. This delayed hyperglycemia is characteristic of the action of epinephrin in the presence of traces of glycogen. As will

[1] It was shown by Page (1923) and confirmed by us that this anesthetic does not raise blood-sugar, and does not interfere with the regulation of blood sugar, as judged by stimulation of the great splanchnic nerve, or by the injection of insulin.

be shown later in this paper, the rise in blood sugar is much more prompt, and is much greater in starved rabbits than in rabbits rendered practically glycogen-free by cold, starvation and strychnine.

TABLE 2

Rabbit 24. Starved for four days at a low temperature. Muscular activity for half an hour immediately before being injected.

April 4.		(4th day of starvation) 0.5 mgm. strychnine sulphate per kilo subcutaneously
April 5.		0.5 mgm. strychnine sulphate per kilo subcutaneously
April 7.	10:30 a.m.	0.5 mgm. strychnine sulphate per kilo subcutaneously
	2:00 p.m.	0.2 mgm. strychnine sulphate per kilo subcutaneously
	4:00 p.m.	0.2 mgm. strychnine sulphate per kilo subcutaneously
April 8.	12:45 p.m.	0.1 mgm. strychnine sulphate per kilo subcutaneously
	4:15 p.m.	0.2 mgm. strychnine sulphate per kilo subcutaneously
		Mild convulsions. Animal hyperexcitable and tremulous. Weight 1140 grams.
	5:45 p.m.	0.125 grams amytal subcutaneously
	6:15 p.m.	Blood sugar 0.099 per cent
	6:40 p.m.	1.76 grams left lobe of liver removed. Glycogen 0.04 per cent (duplicates)
	6:46 p.m.	1 cc. 1 : 1000 epinephrin subcutaneously
	7:59 p.m.	Blood sugar 0.105 per cent
	9:10 p.m.	Blood sugar 0.099 per cent
	10:15 p.m.	Blood sugar 0.113 per cent

TABLE 3

Rabbit 26. Three days' starvation in the cold. Weight at the end of this period, 1830 grams.

March 29	(4th day)	0.45 mgm. strychnine sulphate per kilo subcutaneously
March 31	3:00 p.m.	0.45 mgm. strychnine sulphate per kilo subcutaneously
		Vigorous convulsions
	7:15 p.m.	0.2 gram amytal subcutaneously
	9:35 p.m.	Blood sugar 0.112 per cent
	9:45 p.m.	1.6 grams left lobe of liver removed. Glycogen 0.120 per cent
	9:55 p.m.	1 cc. 1:1000 epinephrin subcutaneously
	10:40 p.m.	Blood sugar 0.163 per cent
	11:07 p.m.	0.5 cc. 1 : 1000 epinephrin subcutaneously
	11:30 p.m.	Blood sugar 0.194 per cent
	11:40 p.m.	Stunned
		Liver, heart and skeletal muscle glycogen-free, i.e., less than 0.00 per cent glycogen

The above experiments may be criticized on two counts. *a*, Amytal may interfere with the hyperglycemic action of adrenalin. *b*, Handling of the liver incident to the removal of a piece for glycogen determination may so upset its glycogenic function as to make the above results unre-

liable. That these criticisms do not obtain, the following experiment shows (table 4).

This rabbit for some unaccountable reason, did not become glycogen-free after starvation and strychnine. The blood sugar previous to administration of epinephrin was 0.270 per cent. It has been our experience to note such high blood sugar in animals that are still under the influence of strychnine. The experiment illustrates that injection of amytal and manipulation of the liver do not prevent epinephrin from causing hyperglycemia.

Epinephrin was now injected subcutaneously into a series of starved rabbits, and the effect on the blood sugar noted. At the end of the experiment the animals were stunned, and the glycogen in the liver estimated.

The injection of epinephrin into starved rabbits constantly evoked

TABLE 4

Rabbit 25. Starved in a cage for three days at room temperature.

March 29	(4th day).	Weight 1370 grams.
	11:00 a.m.	0.61 mgm. strychnine sulphate subcutaneously
		Mild convulsions
	1:40 p.m.	0.171 grams amytal subcutaneously
	4:15 p.m.	2.6 grams left lobe of liver removed. Glycogen 2.07 per cent
	4:35 p.m.	Blood sugar 0.270 per cent
	4:55 p.m.	0.5 cc. 1 : 1000 epinephrin subcutaneously
	5:25 p.m.	Blood sugar 0.353 per cent
	5:48 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	5:55 p.m.	Blood sugar 0.412 per cent
	7:30 p.m.	Stunned
	7:35 p.m.	Liver—0.298 per cent glycogen
		Heart—0.427 per cent glycogen
		Skeletal muscle 0.00 per cent glycogen

hyperglycemia. In two cases at the end of the experiment the liver contained glycogen. In one case it was glycogen-free (table 5).

Epinephrin was now injected into a starved rabbit anesthetized with amytal.

The inference is very strong from these experiments that starving rabbits are never glycogen-free. To test this, two rabbits were starved for four days at room temperature (tables 6 and 6a).

The effect on the blood sugar of repeated injections of epinephrin was now studied, in order to see if, notwithstanding the continuous drain on the glycogen stores, hyperglycemia was constantly obtained. This was found to be the case (table 7). The absence of glycogen may be accounted for by the dyspnoeic state of the animal.

An attempt was now made to see if repeated injections of epinephrin

TABLE 5

Rabbit 1. Weight 2800 grams. Starved three days at room temperature.

5:04 p.m.	Blood sugar 0.142 per cent*
5:20 p.m.	0.58 cc. 1 : 1000 epinephrin subcutaneously
5:43 p.m.	Blood sugar 0.183 per cent
6:00 p.m.	Blood sugar 0.243 per cent
6:20 p.m.	Blood sugar 0.275 per cent, 0.58 cc. 1 : 1000 epinephrin subcutaneously
6:45 p.m.	Blood sugar 0.286 per cent
8:55 p.m.	Blood sugar 0.405 per cent
9:15 p.m.	0.58 cc. 1 :1000 epinephrin subcutaneously
9:35 p.m.	Blood sugar 0.410 per cent (approx.)
9:55 p.m.	Blood sugar 0.407 per cent (approx.)
10:00 p.m.	Stunned
	Liver glycogen = 0.59 per cent

Rabbit 2. Weight 2300 grams. Starved three days at room temperature

5:08 p.m.	Blood sugar 0.149 per cent
5:25 p.m.	0.52 cc. 1 : 1000 epinephrin subcutaneously
5:48 p.m.	Blood sugar 0.227 per cent
6:05 p.m.	Blood sugar 0.290 per cent
6:33 p.m.	Blood sugar 0.337 per cent
6:35 p.m.	0.57 cc. 1 : 1000 epinephrin subcutaneously
7:00 p.m.	Blood sugar 0.331 per cent
9:08 p.m.	Blood sugar 0.446 per cent
9:45 p.m.	Blood sugar 0.407 per cent
	Animal died overnight

Rabbit 3. Weight 3000 grams . Starved 100 hours in the cold

10:45 a.m.	Blood sugar 0.152 per cent
11:00 a.m.	0.6 cc. 1 : 1000 epinephrin subcutaneously
11:57 a.m.	Blood sugar 0.390 per cent
12:01 p.m.	0.6 cc. 1 : 1000 epinephrin subcutaneously
12:29 p.m.	Blood sugar 0.435 per cent (approx.)
12:59 p.m.	Blood sugar 0.500 per cent (approx.)
	Animal allowed to starve an additional 48 hours
1:55 p.m.	Blood sugar 0.112 per cent
1:57 p.m.	0.59 cc. 1 : 1000 epinphrin subcutaneously
2:25 p m.	Blood sugar 0.246 per cent
2:55 p.m.	Blood sugar 0.327 per cent
2:57 p.m.	0.59 cc. 1 : 1000 epinephrin subcutaneously
3:25 p.m.	Blood sugar 0.399 per cent
3.59 p.m.	Blood sugar 0.446 per cent (approx.)
	Animal stunned
	Liver glycogen-free

* We have often observed such high blood sugar in rabbits starved for many days. Its significance is difficult to interpret. Possibly some intermediary metabolite of glucose becomes exhausted after prolonged starvation, resulting in a demand for more blood sugar.

TABLE 5—*Concluded*

Rabbit 4. Weight 2040 grams. Starved 100 hours at room temperature

10:40 a.m.	Blood sugar 0.134 per cent
10:51 a.m.	0.51 cc. 1 : 1000 epinephrin subcutaneously
11:12 a.m.	Blood sugar 0.163 per cent
11:45 a.m.	Blood sugar 0.207 per cent
12:15 p.m.	Blood sugar 0.272 per cent
12:45 p.m.	Blood sugar 0.2915 per cent
12:51 p.m.	0.51 cc 1 : 1000 epinephrin subcutaneously
2:35 p.m.	Blood sugar 0.382 per cent
2:50 p.m.	Stunned
	Liver glycogen 0.37 per cent

eventually led to a deposition of glycogen, as stated by Pollak (1909a). Large doses of this hormone were injected daily into starving rabbits. At the end of from four to nine days the animals were stunned, and the glycogen of the liver, heart and skeletal muscles estimated. A detailed protocol of rabbit 8 is given, and the results for all the rabbits are summarized (tables 8 and 9).

TABLE 6

Rabbit. A. Starved for four days. Weight 1560 grams.

6:15 p.m.	0.150 gram amytal injected subcutaneously
8:00 p.m.	Unconscious
8:10 p.m.	Blood sugar 0.162 per cent
8:15 p.m.	0.75 cc. 1 : 1000 epinephrin injected subcutaneously
9:45 p.m.	Blood sugar 0.352 per cent
10:45 p.m.	Blood sugar 0.74 per cent

TABLE 6a

Rabbits B and C. Starved for four days at room temperature. Stunned, and glycogen content of liver, heart and skeletal muscles estimated:

	GLYCOGEN PERCENTAGE		
	Liver	Heart	Skeletal muscle
B.	0.48	0.23	0.13
C.	0.41	0.19	0.12

These results illustrate that glyconeogenesis to the extent of 1 per cent glycogen can occur under the influence of epinephrin, in a starving rabbit treated also with strychnine. Pollak's figures are much greater, probably because the animals used by him as judged by their weight, were in a better nutritive condition, and because the preliminary treatment in the rabbits used in this investigation was much more thorough. Starvation in the

cold, besides causing a consumption of glycogen, also causes a depletion of glycogen precursors.

TABLE 7

Rabbit 6. Weight 2500 grams. Starvation began January 9. Temp. 0°C.

Jan. 9		0.5 mgm. strychnine sulphate subcutaneously
Jan. 10		0.8 mgm. strychnine sulphate subcutaneously
Jan. 11 a.m.		0.8 mgm. strychnine sulphate subcutaneously
Jan. 11 p.m.		0.8 mgm. strychnine sulphate subcutaneously
		A control rabbit died of strychnine convulsions
Jan. 12.	11:00 a.m.	Blood sugar 0.112 per cent
	11:05 a.m.	0.5 cc. 1 : 1000 epinephrin subcutaneously
	11:35 a.m.	Blood sugar 0.229 per cent
	12:15 p.m.	Blood sugar 0.305 per cent. Urine contains trace of glucose
	12:37 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	1:10 p.m.	Blood sugar 0.372 per cent
	2:30 p.m.	Blood sugar 0.448 per cent (approx.)
	4:00 p.m.	Blood sugar 0.415 per cent (approx.)
Jan. 14.	7:00 a.m.	Blood sugar 0.129 per cent, 0.5 cc. 1 : 1000 epinephrin subcutaneously
	8:30 a.m.	Blood sugar 0.235 per cent
	8:35 a.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	9:40 a.m.	Blood sugar 0.342 per cent
	11:40 a.m.	Blood sugar 0.3775 per cent
	12:20 p.m.	1.0 cc. per cent 1:1000 epinephrin subcutaneously
Jan. 15.	1:00 p.m.	Blood sugar 0.133 per cent
	4:10 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	4:50 p.m.	Blood sugar 0.199 per cent
	5:00 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	7:00 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	10:00 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
Jan. 16.	4:00 p.m.	Blood sugar 0.122 per cent
	4:05 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	5:05 p.m.	Blood sugar 0.228 per cent
	5:15 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	6:10 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	7:30 p.m.	Blood sugar 0.305 per cent
	8:00 p.m.	1.0 cc. 1 : 1000 epinephein subcutaneously
	9:30 p.m.	Blood sugar 0.305 per cent
	9:35 p.m.	1.0 cc. 1 : 100 epinephrin subcutaneously
	12:45 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
Jan. 17.		1.0 cc. 1 : 1000 epinephrin subcutaneously
Jan. 18.		Animal weak and dyspnoeic. Stunned
		Liver, heart and skeletal muscle contain less than 0.009 per cent glycogen

The possibility of demonstrating greater glyconeogenesis by injecting insulin along with the adrenalin was next investigated. The results are shown in table 10.

The percentage of glycogen in the liver in two of the cases was greater than in rabbits injected with epinephrin only, whereas the amount in skeletal muscle in all cases was less. Insulin evidently acts by hindering epinephrin glycogenolysis.

It is difficult to explain why a starving rabbit injected with epinephrin, particularly with epinephrin and insulin, should deposit glycogen. It is

TABLE 8

Rabbit 8. Medium-sized rabbit. Starved for five days at 0°C. Given 0.45 mgm. strychnine sulphate per kilo on the fifth day.

Jan. 21.	(sixth day of starvation)	
	4:50 p.m.	0.5 cc. 1 : 1000 epinephrin subcutaneously
	8:25 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	10:20 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	12:55 a.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
Jan. 22.	6:00 p.m.	0.5 cc. 1 : 1000 epinephrin subcutaneously
	9:10 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	11:15 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	12:15 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
Jan. 23.	1:30 p.m.	0.5 cc. 1 : 1000 epinephrin subcutaneously
	3:45 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	4:45 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	5:45 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	8:05 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	10:45 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	4:00 a.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
Jan. 24.	3:30 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	5:00 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	6:30 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	9:30 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	11:40 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	1:05 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
Jan. 25.	1:45 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	5:30 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	7:10 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	9:10 p.m.	1.0 cc. 1 : 1000 epinephrin subcutaneously
	11:15 p.m.	Stunned. Liver 0.98 per cent glycogen
		Heart 0.44 per cent glycogen
		Skeletal muscle 0.19 per cent glycogen

Sugar in blood from heart at time of death, 0.82 per cent. Urine expressed from bladder gave no reduction with Benedict's solution

probable that injection of epinephrin causes glyconeogenesis as well as glycogenolysis, the newly formed liver glycogen being decomposed again into glucose. The relative extent of each of these processes will determine the amount of glycogen actually found. After repeated injections very little of the glucose appears in the urine, because of a raised renal threshold

for this substance. Under these conditions formation will exceed glycogen breakdown.

Another possibility is a change in the character of the glycogen. It must be remembered that glycogen can not be considered as a chemical entity, but rather as a mixture of polysaccharides, which gives a red color

TABLE 9

Glycogen content of rabbits injected repeatedly with epinephrin

RABBIT NUMBER	DATE	TOTAL 1:1000 EPINEPHRIN INJECTED AT INTERVALS DURING DAY	GLYCOGEN			PRELIMINARY TREATMENT
			Liver	Heart	Muscle	
		cc.	*per cent*	*per cent*	*per cent*	
7	January 21	3.5				Starvation in the cold for five days. Strychnine convulsions
	22	3.5				
	23	6.5				
	24	6.0				
	25	3.0	0.331	0.536	0.141	
28	April 1	1.5				Starvation for four days. Strychnine convulsions on two successive days
	2	4.2				
	3	6.4				
	4	5.6				
	5	5.0				
	7	8.0				
	8	6.0	0.161	0.234	0.133	
8	January 21	3.5				Starvation in the cold for five days. Strychnine convulsions
	22	3.5				
	23	6.5				
	24	5.5				
	25	4.0	0.98	0.44	0.19	
22	February 22	3.5				Starvation in the cold for five days. Strychnine convulsions
	23	3.0				
	25	5.0				
	26	4.0				
	27	5.0				
	28	5.0				
	29	6.0				
	March 1	5.0				
	2	1.0	1.05	0.100	0.265	

with iodine, which resists boiling with 30 per cent KOH, and is insoluble in certain concentrations of ethyl alcohol. Neubauer (1909) found that rabbits poisoned with phosphorus deposited glycogen in the liver if fed levulose or sucrose, but not if fed glucose. Glycogen deposited in the liver after feeding rabbits with levulose was found by Pollak (1909b) to be more

TABLE 10

Glycogen content of starving rabbits injected repeatedly with epinephrin and insulin

RABBIT NUMBER	DATE	TOTAL 1:1000 EPINEPHRIN INJECTED EACH DAY	INSULIN INJECTED	GLYCOGEN			PRELIMINARY TREATMENT
				Liver	Heart	Muscle	
		cc.	*units*	*per cent*	*per cent*	*per cent*	
11	January 27	0.5	2.5				Starvation in the cold for three days. Strychnine convulsions
	28	2.8	5.0				
	29	2.0	2.0				
	30	4.25	2.0				
	31	5.0	1.75				
	February 1	7.0	1.5				
	2	4.25	1.5				
	3	8.0	1.0	0.345	0.315	0.063	
12	January 30	4.0	2.25				Starvation in the cold for four and a half days. Strychnine convulsions
	31	5.0	1.75				
	February 1	7.0	1.5				
	2	4.25	1.5	1.67	0.104	0.048	
13	January 30	4.0	2.25				Starvation in the cold for four and a half days. Strychnine convulsions
	31	5.0	1.75				
	February 1	7.0	1.5				
	2	4.25	1.5				
	3	7.5	1.5				
	4			0.152	0.160	0.107	
14	January 30	4.0	2.25				Starvation in the cold for four and a half days. Strychnine convulsions
	31	5.0	1.75				
	February 1	7.0	1.5				
	2	4.25	1.5				
	3	7.5	1.5				
	4			2.77	0.304	0.052	
15	February 9	2.5	0.75				Starvation for five days. Strychnine convulsions. Then additional starvation for two and a half days
	10	5.5	1.5				
	11	7.0	1.5				
	12	8.0	2.5				
	13	5.0	1.5				
	14	4.0		1.45	0.039	0.00	
16	February 8	2.7	2.0				Starvation for five days. Strychnine convulsions
	9	4.7	2.1				
	10	6.5	1.5				
	11	7.0	1.5				
	12	8.0	1.5				
	13	5.0	1.5				
	14	4.0		1.27	0.392	0.130	

TABLE 10—*Concluded*

RABBIT NUMBER	DATE	TOTAL 1:1000 EPINEPHRIN INJECTED EACH DAY	INSULIN INJECTED	GLYCOGEN			PRELIMINARY TREATMENT
				Liver	Heart	Muscle	
		cc.	*units*	*per cent*	*per cent*	*per cent*	
18	February 22	2.0	0.5				Starvation for five and three-quarter days. Strychnine convulsions
	23	3.0	1.0				
	25	5.0	1.0				
	26	4.0	1.0				
	27	5.0	1.0				
	28	5.0	1.0				
	29	6.0	1.0				
	March 1	5.0	1.0				
	2	1.0		1.09	0.178	0.096	
19		Ditto		0.516	—	—	Ditto
27	April 1	1.5	1.0				Starvation for six days. Strychnine convulsions on fifth and sixth days
	2	4.2	1.0				
	3	6.4	1.0				
	4	5.6	1.0				
	5	1.0	1.0				
	7	8.0	1.0				
	8	6.0	1.0	0.031	0.095	0.00	
29	April 7	3.5	1.0				Starvation for six days. Strychnine convulsions on fifth and sixth days
	8	5.6	1.0				
	9	6.3	1.0				
	10	8.0	1.0				
	11	5.0	1.0				
	12	4.0	1.0	1.12	0.406	0.067	

resistant to small doses of epinephrin than that formed out of glucose. From time to time statements appear in the literature that depancreatized dogs deposit glycogen in the liver when fed levulose. Such observations suggest that glycogen may vary in properties, depending on its source.

That glycogen may accumulate in the liver of a starving organism to the extent of nearly three per cent is a paradox in the light of the common conception of this substance as a storage material. The observation suggests that liver glycogen is an essential step in the transformation of fat or protein to carbohydrate. When this transformation cannot take place, as after phosphorus poisoning, or liver extirpation, the blood sugar falls and the organism dies unless glucose is supplied to it.

Regarded purely from the teleological viewpoint, it is not likely that the organism would tolerate such an enormous breakdown of its tissue protein as to permit the liver to deposit a great quantity of glycogen. It is

well known that the fat stores are the most important source of energy in starvation. The lipemia and fatty infiltration of the liver which occur in this condition may be due to the rush of fat to the liver to form glycogen. It is possible that fat is not oxidized as such, but as glucose. The low respiratory quotient, which accompanies "oxidation" of fat would then be due to an algebraic summation of two factors: *a*, Transformation of fat into carbohydrate, which would lead to a diminution of the R.Q.; *b*, oxidation of carbohydrate, which would tend to increase it.

The contention that endogenous fat cannot form carbohydrate is supported chiefly by experiments on respiratory exchange and D/N ratios of diabetic and phlorhizinized dogs. These experiments are not conclusive because the calculations are based on several unproven assumptions, chief of which is inability of such animals to oxidize glucose.

The experiments performed by us are not suitable for calculations based on nitrogen exchange. Such large doses of epinephrin bring too many factors into play to permit the formation of any valid conclusions.

CONCLUSIONS

The injection of epinephrin into starved rabbits always evokes extensive hyperglycemia, even when the injections are kept up for days.

When the liver is glycogen-free, injection of epinephrin does not lead to hyperglycemia. When the liver contains traces of glycogen, hyperglycemia of delayed onset ensues, the extent being proportional to the amount of glycogen.

Mere starvation does not make rabbits glycogen-free. Starvation in the cold, followed by strychnine convulsions will make the liver, heart and muscles glycogen-free for several hours.

Repeated injection of epinephrin into starved, strychnine-treated rabbits permits glycogen to accumulate in the liver, heart and muscles.

When insulin is given to starved strychnine-treated rabbits along with epinephrin, considerable quantities of glycogen, up to 2.77 per cent accumulate in the liver.

The above experiments, together with those quoted from the literature, demonstrate that glycogen is an obligatory, and not an optional step in the formation of blood sugar from the non-carbohydrate stores of the organism.

It is a pleasure to express my indebtedness to Prof. J. J. R. Macleod for directing this research, and for criticizing this manuscript.

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OBSERVATIONS ON DEPANCREATISED DOGS BEFORE AND AFTER THE WITHDRAWAL OF INSULIN

I. L. CHAIKOFF, J. J. R. MACLEOD, J. MARKOWITZ AND W. W. SIMPSON

From the Physiological Laboratory, University of Toronto

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It is still an open question whether the essential abnormality of function in diabetes is due to the tissues having lost the power to metabolize glucose, or to an over-production of glucose. The advent of insulin has placed in our hands a new means of attack on this problem, since it enables us to keep depancreatised animals under much more favorable conditions than previously was the case. The following is a report of some observations on the relationship existing in depancreatised dogs with and without insulin between the metabolism of sugar, on the one hand, and that of fat, ketone bodies, nitrogen and phosphoric acid on the other.

For many years there have been two schools with regard to the origin of the sugar in the starving diabetic animal. One of these founded by O. Minkowski, and now led chiefly by Lusk, holds that protein is the chief if not the sole source of this sugar. The other school, represented by von Noorden, and more recently by Geelmuyden (1923), maintains that fat as well as protein is its mother-substance. Many of the experiments designed to test these possibilities have been made on depancreatised or phloridzinized animals, which, since they are glycogen-free, are in a highly unphysiological state and may not represent the true conditions in the normal animal. Moreover, the calculations upon which many of the conclusions depend often postulate certain unproven assumptions, chief of which is inability of the organism to consume glucose. As judged by experiments on isolated, surviving organs (Patterson and Starling, 1913) (Landsberg, 1914), and by experiments on eviscerated dogs (Macleod and Pearce, 1913, 1914), the tissues still possess this power. In using the respiratory quotient it must be recalled that this is the resultant of a number of factors. Thus, an R.Q. of 0.71 might be the algebraic summation of: *a*, formation of sugar from fat, which would tend to diminish it; *b*, oxidation of glucose, which would tend to raise it. These two processes operating to a variable extent in different experiments may possibly explain the somewhat divergent results reported by various investigators regarding the R.Q. of starving diabetic dogs. In a similar manner the D:N ratio is the momentary resultant of an equilibrium between glucose production, utili-

zation and excretion, on the one hand, and nitrogen excretion on the other. It is not surprising, therefore, that different phloridzinized dogs should not have the same D:N ratio, the range usually being between 2.6 to 4.0 (Loewe, Ringer and Lusk, 1923), a fact which is not in harmony with the exclusive derivation of sugar from protein. Many have observed that emaciated, starving, totally depancreatized dogs often give a low D:N ratio which may be even less than unity, cachexia, starvation and various obscure conditions often being associated with these low ratios, and thus suggesting the possibility that the depletion of the fat stores is co-related with a fall in sugar excretion. Allan's experiments on partially depancreatised dogs support this explanation. Such animals could be kept in good health and sugar-free if undernourished, but eventually showed copious glycosuria and coma if they were fattened (Allen, 1916–17). It would seem probable from these considerations that the D:N ratio is not dependent solely on the breakdown of protein to form glucose, but is the resultant of a number of conditions which, averaged over many days, give the figure of about 2.8:1.

Although the results which we have so far obtained do not justify a categorical denial of the usually accepted theory that the essential abnormality in diabetes is lessened utilization of glucose of the tissues, we are placing them on record, since they bring to light some facts of importance in connection with the problem as a whole.

The observations here recorded consist of three divisions: 1, A comparison of the amounts of sugar, ketone bodies, fat, and phosphoric acid, in the blood of fat and lean starving diabetic dogs, after discontinuing injections of insulin for several days; and 2, the time relationships of the changes that occurred in these substances when insulin was again given. 3, The D:N ratios of depancreatised dogs after withdrawal of insulin.

1. SUGAR, PHOSPHATES AND KETONE BODIES OF BLOOD IN FAT AND THIN DIABETIC DOGS. Five depancreatised dogs (see table 1) kept alive by means of insulin were used in these experiments. The insulin was discontinued for several days before the blood was removed for analysis. The designations "fat," "thin" and "emaciated" indicate the general nutritive condition of the animals. The fattening of depancreatised animals can usually be brought about by giving them large quantities of cane sugar along with insulin. All the dogs have died since these observations were completed and at post-mortem examination no visible pancreas could be seen outside the duodenum in any of them, except in dog B, in which a small nodule of pancreatic tissue was detected near the main duct. On examination of serial sections of the duodenum, nodules of pancreatic tissue were also seen in dogs B and M. No islet tissue has been detected in these nodules and a full account of this part of the work will be published shortly by D. J. Bowie. It may be mentioned here that we have occasionally found similar

TABLE I

The chemical composition of the blood in starving, fat and thin diabetic dogs after discontinuing insulin

DOG	DATE	NUTRITIVE CONDITION	INSULIN LAST ADMINISTERED	CONDITION AT TIME OF EXPERIMENT	BLOOD SUGAR	INORGANIC PHOSPHOR. PER 100 CC.	B-OXYBUTYRIC ACID PER CUBIC CENTIMETER	ACETOACETIC ACID PER CUBIC CENTIMETER	FAT PER CUBIC CENTIMETER
					per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*
A	October 15	Thin	3 days ago	Excellent	0.380	5.0	0.27*	0.044*	18.6
	November 20	Emaciated	6½ days ago	Slightly asthenic	0.359		0.070	0.064	
	December 4	More emaciated	6 days ago	Vomiting,† weak and tottering. Dehydrated. Blood from heart. 16 units insulin. Convulsions. Died	0.423	4.2	0.21	0.12	
B	November 7	Fat	3 days ago	Slightly asthenic	0.438	6.4	0.23	0.32	10.3
	November 28	Somewhat fatter	5 days ago	Vomiting, weak and tottering. Dehydrated. Tension in eyeballs low. Dry tongue	0.600	4.98	0.41	0.86	9.5
	October 30	Same as above	3 days ago	Slightly asthenic	0.700	5.89		0.53	11.7
G		Very fat	2½ days ago	Slightly asthenic. No respiratory embarrassment. Died overnight	1.38	8.3	0.70	1.5	15.0

M		Emaciated	No insulin on and off for 2 weeks. Last dose 4 days ago	State of partial collapse	0.468		0.48	0.45	5.5
T		Medium	3 days ago	Slightly asthenic			0.12	0.063	

* Calculated as acetone (Marriot's method, 1914).

† The symptoms vomiting, asthenia, dehydrated appearance mentioned above are very common in starved depancreatized dogs deprived of insulin. The tongue is dry, the tension in the eyeballs is low, respirations are rapid. It was thought on one occasion that the breath smelt of acetone. Unless insulin is given promptly, these animals die, and even with prompt measures it has not been possible to save them all.

nodules of pancreatic tissue in the duodenal wall of normal (non-depancreatized) dogs. The following conclusions seem to be warranted: 1.

TABLE 2a

Dog. A. Thin dog. Depancreatised 6 weeks ago. Last dose of insulin 3 days ago.

TIME (OCTOBER 15, 1924)	BLOOD ANALYSIS					
	Blood sugar	Inorganic phosphorus per 100 cc.	B-oxybutyric acid per cubic centimeter	Acetoacetic acid per cubic centimeter	Blood fat per cubic centimeter	Hemoglobin
	per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
10:18	0.380	5.0	0.27	0.044	18.6	100
10:42	20 units insulin					
11:22	0.336	2.9	0.13	0.034	21.2	—
11:53	0.235	2.1	0.089	—	20.0	102
12:25	0.152	1.74	0.095	0.015	14.9	100
1:00	0.119	1.63	0.081	0.030	19.3	97
2:07	0.074	1.63	0.11	0.029	18.3	94.5
3:10	0.079	1.92	0.12	0.045	14.9	87
4:15	0.084	2.18	0.12	0.030	17.3	100

TABLE 2b

Dog B. Red fat retriever. Last dose of insulin 3 days ago. Starving. Depancreatised 3 weeks ago.

TIME (OCTOBER 30)	BLOOD ANALYSIS					
	Blood sugar	Inorganic phosphorus per 100 cc.	B-oxybutyric acid per cubic centimeter	Acetoacetic acid per cubic centimeter	Blood fat per cubic centimeter	Hemoglobin
	per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*per cent*
10:30	0.700	5.89		0.53	11.7	100
10:33	25 units insulin					
10:44	0.653	5.38		0.53	13.9	105
11:18	0.430†	4.55			14.7*	—
12:04	0.420†	4.00		0.43	11.0	102.5
1:25	0.390	4.50		0.33	11.8	—
3:00	0.316	—		0.24	12.0	108
4:07	0.315	—		0.077	11.2	108
5:10	0.291	5.16		0.071	9.8	—

* Approximately.

† Duplicates checked to 1½ per cent.

Note. B-oxybutyric acid determinations not satisfactory due to formation of a yellow color in most of the final distillates. The figures were definitely lower than the corresponding acetoacetic acid determinations.

The fasting blood-sugar in thin diabetic dogs is decidedly lower than in fat ones, the extreme ranges being 0.359 per cent in an emaciated dog

(dog A, November 20), and 1.38 per cent in a very fat one (G). 2. The ketone bodies are also higher in fat as compared with thin dogs. In fat dogs acetoacetic acid is relatively more plentiful than B-oxybutyric and in thin ones oxybutyric is relatively more plentiful than diacetic. In one emaciated dog (M) the concentration of both acids was about equal. 3. The concentration of blood fat, and of inorganic phosphate is not evidently dependent on the nutritive condition of the animal. The phosphates in both types of animal are above the percentage found in normal dogs.

Discussion. It is of course possible, although not likely, that the observed differences in the blood sugars may have been due to disparity in the glycogen content of the liver in thin and fat dogs respectively. Since most of the animals died during the night it was useless to determine the

TABLE 2e

Dog B. Red fat retriever. Completely depancreatised 1 month before experiment. Last dose of insulin 3 days ago. Starving.

TIME (NOVEMBER 7)	BLOOD ANALYSIS					
	Blood sugar	Inorganic phosphorus per 100 cc.	B-oxybutyric acid per cubic centimeter	Acetoacetic acid per cubic centimeter	Blood fat per cubic centimeter	Hemoglobin
	per cent	*mgm.*	*m.m.*	*m.m.*	*mgm.*	*per cent*
10:20	0.438	6.4	0.23	0.32	10.3	100
10:22	50 units insulin					
10:46	0.408	5.96	0.16	0.38	10.6	108.5
11:23	—	—	0.15	—	11.5	—
11:40	0.289	3.13	0.13	0.35	12.6	100
12:10	0.218	2.63	0.065	0.19	10.6	95
12:43	0.060	2.24	0.067	0.20	10.9	97
1:58	0.090	2.60	0.058	0.18	11.6	94.5
3:06	0.105	3.70	0.061	0.14	11.6	93

glycogen, but we are at present collecting data which should enable us to check this possibility. In any case we consider the approximate parallelism between the amount of visible fat in the body and the height of the blood sugar as very suggestive of a relationship between the two. It is also of significance that few depancreatised animals that have been kept alive by means of insulin can survive its withdrawal for more than five days, fat ones being much more serious risks in this regard than emaciated ones. The fatal symptoms in fat animals are very like those of severe coma in man. The fact that critical symptoms supervene in fat diabetic dogs more quickly than in thin ones on withdrawal of insulin corroborates the clinical experience, that diabetic patients who have put on much fat under insulin are liable to develop alarming acidosis when its use is discontinued.

In tables 2a, 2b and 2c, and in figures 1 and 2 are shown the effects of

again administering insulin to diabetic animals from which it had been withheld for several days.

Observations. Within one-half hour after the injection of insulin, there was a definite fall in blood sugar, B-oxybutyric acid and inorganic phos-

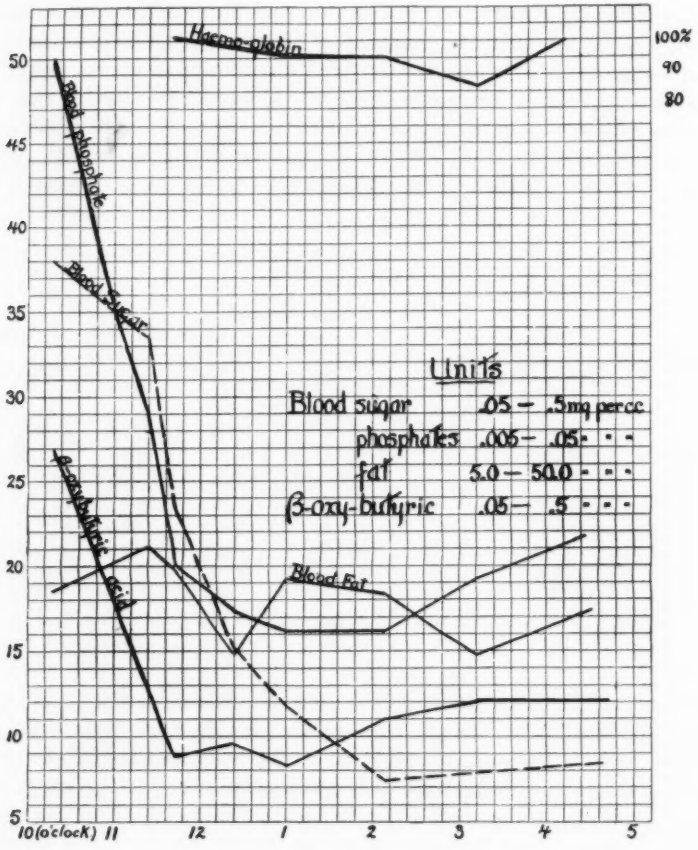

Fig. 1

phate, the fall in B-oxybutyric acid and inorganic phosphate running practically parallel with the blood sugar. The inorganic phosphorus began to rise again before the blood sugar. The B-oxybutyric acid rose with the phosphorus in one of the experiments but not in another. The acetoacetic acid diminished more slowly than the oxybutyric acid, and did not

begin to rise again in any of the observations. Blood fats were unchanged beyond experimental error. The percentage of hemoglobin did not show any significant changes.

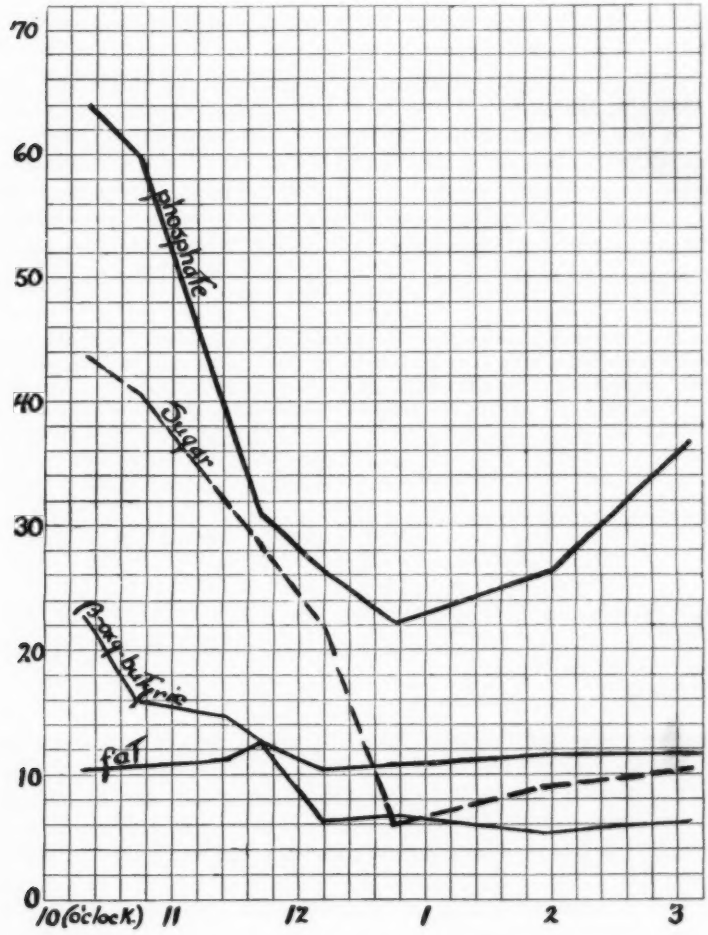

Fig. 2

Discussion. The striking parallelism in the fall of blood sugar, inorganic phosphate and B-oxybutyric acid, suggests that they may go to form a common product. It would seem that the phosphate of this compound is turned loose again when the organic portion of it is oxidised. The findings

do not conflict with the view recently championed by Geelmuyden that acetone bodies are intermediary products in the breakdown of fats to form sugar, and that the transformation is incomplete in the absence of insulin as is the case in the diabetic organism.

In table 3 are shown the changes in the sugar, ketone bodies, phosphorus and fat in the blood of a depancreatised dog that had been kept for a month with insulin after pancreatectomy. The blood-sugar fell during two days following the withdrawal of insulin, and then suddenly increased on the third day to the unprecedented high level of 1.38 per cent. Although the animal seemed to be sick on the evening of this day, it was not considered necessary to give insulin to save it. It died, however, during the night. It is probable that the fall in sugar during the two days ran parallel with the disappearance of glycogen from the liver, for the animal had been given large amounts of sugar during the insulin treatment. The phosphates,

TABLE 3

Dog E. Black and tan large dog. Depancreatised December 23, 1924. Fattened up after the operation with large quantities of meat and sugar, and injections of insulin. Gained over 3 pounds during this period, weighing 26 lbs. 7 oz. at beginning of experiment. Last dose of insulin and food morning of January 20.

DATE	TIME	BLOOD SUGAR	INORGANIC PHOSPHOR. PER 100 CC.	B-OXYBUTYRIC ACID PER CUBIC CENTIMETER	ACETOACETIC ACID PER CUBIC CENTIMETER	BLOOD FAT PER CUBIC CENTIMETER
		per cent	*mgm.*	*mgm.*	*mgm.*	*mgm.*
January 21	10:30 a.m.	0.637	6.7	0.064	0.058	12.0*
	11:40 p.m.	0.540	6.7	0.26	0.26	
January 22	12:10 p.m.	0.502	7.5	0.49	0.86	15.0
January 23	1:30 a.m.	1.38	8.3	0.70	1.5	

Dog found dead at 8 a.m.

* Duplicates.

the ketone bodies and the blood fat all increased, that of acetoacetic acid most rapidly. It will also be observed when the increase in each case is computed in milligrams per hour that it was fairly steady for B-oxybutyric acid, but became progressively greater in that of acetoacetic acid being 0.15 mgm. per 100 cc. during January 21, 0.25 on January 22 and 0.35 on January 23.

2. THE D:N RATIOS OF DEPANCREATISED DOGS AFTER WITHDRAWAL OF INSULIN. In various investigations on depancreatised dogs carried on in this laboratory during recent years we have found it unusual to obtain D:N ratios of 2.8 as described by Minkowski. When the animals were being given considerable quantities of meat, ratios at or approaching this value were not uncommon, but they were usually much lower when food was withheld. Since so much emphasis has been placed on the

significance of these ratios in connection with the derivation of sugar in diabetes, we decided that it would be important to study them in depancreatised dogs, after the withdrawal of insulin, the advantage here being that animals which were either fattened or kept lean could be used. For reasons already given, however, the observations on the former type of

TABLE 4a

Dog M. Small, thin fox-terrier. Completely depancreatised on January 22, 1925. Food and insulin withdrawn March 12.

24-HOUR PERIOD ENDING MORNING OF:	GLUCOSE	NITROGEN	D:N	REMARKS
	grams	*grams*		
March 13	15.5	1.37	11.3	
March 14	18.1	1.37	13.2	
March 15	7.5	1.56	4.80	
March 16	4.23	1.90	2.23	
March 17	5.01	2.38	2.10	
March 18	5.82	3.03	1.96	
March 19	38.6	3.12		39 grams Bactodextrose given per os*
March 20	5.67	4.15	1.37	
March 21				Animal given insulin. Fed
March 22	Animal died. Post mortem revealed no pancreatic fragments			

* Equivalent to 35.7 grams glucose.

TABLE 4b

Dog L. Thin dog. Part hound, part fox-terrier. Complete pancreatectomy February 25. Insulin and food withdrawn March 17 (morning).

24-HOUR PERIOD ENDING MORNING OF:	GLUCOSE	NITROGEN	D:N	REMARKS
	grams	*grams*		
March 20	7.75	2.88	2.79	
March 21	—	—		
March 22	—	—		
March 23	6.83	3.48	1.91	
March 24	53.7	4.3*		39 grams Bactodextrose per os
March 25	4.55	3.00	1.52	

* Duplicate.

animals have proved to be very difficult, because of the fatal issue from coma which usually results from withdrawal of insulin. The ratios on three thin animals are given in tables 4a, 4b, and 4c.

In the animal of 4a food and insulin were withdrawn on the day preceding the beginning of the observations and it will be seen that the excre-

tion of nitrogen steadily rose from day to day, whereas that of glucose remained high until between the third and fourth days, when it suddenly fell to a lower level, after which it remained fairly constant. As a consequence the D:N ratio was very high for the first three days, fell suddenly on the fourth and steadily thereafter, being below 2.8 for the last five days

TABLE 4c

Dog. Pug. Boston bull. Weight 10 pounds. Depancreatised February 26. Good recovery, the wound healing by first intention. Food and insulin withdrawn on morning of March 11.

24-HOUR PERIOD ENDING MORNING OF:	TOTAL N	TOTAL GLUCOSE	D:N	REMARKS
March 13	2.76	14.0	5.08	
March 14	3.12	40.1	—	39 grams Bactodextrose given by stomach tube
March 15	3.08	4.41	1.43	
March 16	2.88	2.62	0.91	
March 17	2.54	34.8	—	39 grams Bactodextrose (36.6 grams glucose) given by stomach tube. Animal weak
March 18				Died suddenly at 1:30 p.m.

Post mortem showed no pancreatic remnants. Section of duodenum revealed no pancreas in the duodenal wall.

TABLE 4d

Dog A. Pancreas removed. Food and insulin withdrawn November 14.

DATE	WEIGHT	TOTAL N (URINE)	GLUCOSE EXCRETED	D:N	BLOOD-SUGAR
		grams	*grams*		
November 15	15 pounds 6 ounces	5.10	45.96	9.012	0.410
November 16	15 pounds 1 ounce	4.61	15.86	3.442	—
November 17	14 pounds 13 ounces	6.29	21.42	3.405	0.333
November 18	14 pounds 4 ounces	6.42	20.02	3.118	0.312
November 19	13 pounds 7 ounces	5.92	17.16	2.898	0.357
November 20	13 pounds 0 ounce	5.665	14.90	2.631	0.308

Remarks. Dog passed no feces during this period. Scabbiness of skin, previously present, disappeared in absence of food and insulin. Latter urine samples green—but no bile pigment present.

of the observation. On the eighth day, 36 grams of glucose were given by mouth. This caused no increase in the respiratory quotient (details to be published later) and 38.6 grams of glucose were excreted. Calculating from D:N ratio of the previous day, 6.1 grams of this may have come from protein, leaving 32.5 as derived from the injected sugar.

In the animal of 4b in which three days were allowed to elapse after

withholding food and insulin, the D:N ratio was at 2.79 on the fourth day and fell to 1.9 by the seventh. Ingestion of 36 grams of glucose on the eighth day failed to increase the R.Q. and caused 53.7 grams to appear in the urine; of this, 8.2 grams may have come from protein (according to the D:N ratio of the previous day) so that 45.5 grams were derived from some other source than the ingested sugar or protein. The D:N ratio for this day after allowing for the ingested glucose was, therefore, $53.7 - 36 = 17.7:4.31$ or 4.1. As to whether this means that the proportion of glucose derived from protein was larger than that usually assumed (50 per cent) or that fat was its source, we are not prepared to say.

In the animal of 4e the main fact of interest is that on both occasions on which glucose was given a considerable proportion of it was apparently utilized, although we are perfectly certain that no trace of pancreatic tissue remained in the body. We do not, however, place too much weight on this experiment, since the glucose was either given early, when the D:N ratio of the previous day was still influenced by the sugar derived from

TABLE 4e

Dog M. Pancreas removed one month ago. Food and insulin withdrawn January 7 and January 16.

DATE	URINE SUGAR	URINE NITROGEN	D:N	
	grams	*grams*		
January 9	10.56	5.208	2.028	
January 10	0.515	3.496	—	
January 18	2.160	1.535	1.407	
January 19	2.640	1.593	1.65	
January 20	1.010	1.708	1.12	Dog died

stored glycogen, or late, when the animal was evidently near its end, since it died on the succeeding day. In the animal of 4d the D:N ratio was measured from the day after the withdrawal of insulin, and it will be observed, that from that time until the end of the observation on the 7th day the D:N ratio steadily fell finally reaching to a level slightly below 2.8. The excretion of nitrogen rose after a temporary initial fall. It rose markedly on the 4th day and thereafter slowly declined. The results in this animal correpond closely to those reported as typical by Minkowski.

In the animal of 4e the D:N ratio was determined for 2 days immediately after removal of insulin and food, then again for 3 days a week later. It can be seen that the D:N ratio was far below the Minkowski level both in the early and late stages of the observation.

We are certain that pancreatectomy was complete in all cases so that we do not consider that this ratio can be given much weight in drawing conclusions as to the source of sugar in the diabetic organism.

SUMMARY

1. The percentage amounts of sugar and ketone bodies were found to be decidedly higher in fat depancreatised dogs several days after discontinuing insulin and food than in thin ones, but there was no constant difference in the amounts of fat or phosphoric acid.

2. Of the ketone bodies of the blood, acetoacetic acid was relatively greater in the fat dogs, and oxybutyric acid in the thin ones.

3. Fat animals survived the withdrawal of insulin for a much shorter period of time than thin ones, and they succumbed to symptoms not unlike those of diabetic coma in man.

4. On giving insulin again, the sugar, the inorganic phosphates and the oxybutyric acid diminished at uniform rates. Acetoacetic acid fell more slowly and the fat not at all, within several hours. After the insulin effect had passed off, the phosphoric acid rose first.

5. The D:N ratio declined steadily in thin animals following the withdrawal of insulin and food, to far below the 2.8 level.

6. In two out of three animals, ingestion of glucose was followed by the reappearance of most of it in the urine, but in the remaining one considerably more than the ingested amount appeared.

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STUDIES ON THE PHYSIOLOGY OF THE LIVER

XI. The Extrahepatic Formation of Bilirubin

FRANK C. MANN, CHARLES H. SHEARD AND JESSE L. BOLLMAN

From the Division of Experimental Surgery and Pathology, The Mayo Foundation, and the Section on Physics, Mayo Clinic, Rochester, Minnesota

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We have reported in previous papers that in the urine, plasma and fat of totally dehepatized dogs, a yellow pigment appears which gives a positive reaction to all the accepted chemical tests for bilirubin. This seemed definite and conclusive evidence that the liver is not essential for the formation of bile pigment. However, the results as previously presented are open to two objections: 1, the chemical tests for bilirubin are not considered absolutely specific; and 2, since our methods for total extirpation of the liver require preliminary operations to force the development of a collateral circulation for the return of the blood from the lower extremities and viscera, these operations and changes in circulation may in some fashion be responsible for the appearance of the yellow pigment after total hepatectomy (McNee).

The accepted tests for bilirubin are not considered absolutely specific as a few other substances will give a positive reaction with the same reagents, but in general these substances are not normal constituents of the blood. It would seem, therefore, that while a substance other than bilirubin might occur in the blood which would give the reaction for bilirubin, the chemical identification of the pigment is practically definite. The possibility must also be considered that there may be substances intermediate between hemoglobin and bile pigment as found in the bile, which would give positive reactions with the chemical tests for bilirubin. It is possible that these intermediate substances are formed outside the liver, but the elaboration of the pigment into its final form occurs only in the liver. All of our observations are opposed to these considerations and tend to prove that the pigment which accumulated in the blood of the dehepatized dog was the same as the pigment found in the bile. Since chemical tests might be open to question, only the physicochemic method of identification of the pigment remained. The curve of light transmission of the pigment appearing in

the dehepatized animal was compared to the curve produced by bile pigment.

The objection that the pigment might be produced in consequence of the preliminary operations necessary before total extirpation of the liver, has been met by the development of a method of removing the liver with the other abdominal viscera which does not necessitate a preliminary operation.

In this connection we wish to point out that we have frequently controlled our results of the effect of hepatectomy by the removal of the liver in animals in which the blood supply to the organ had not been previously decreased. It should be emphasized that any such method will of necessity have to maintain the venous return from the lower extremities and be complicated by a loss of all the organs drained by the portal vein. At first we employed the method of performing a reverse Eck fistula, and later, after the collateral for returning the blood from the lower limbs had become established, removing all the abdominal viscera. In the beginning we employed the animals in which a collateral sufficient to allow the ligation of the portal vein did not develop. As pointed out in a previous paper (Mann, Bollman and Magath, 1924), in such animals the circulation through the liver was increased before the final removal. Later we forced a collateral to develop for the venous return of the lower limbs by ligating the vena cava between the entrance of the lumbo-adrenal veins and the hepatic veins as we suggested in a previous paper (Mann and Magath, 1924). However, as the complete acute occlusion of the vena cava at this site frequently produced death before a collateral could be established, we later employed the method of partial occlusion of the vena cava. This method proved very satisfactory, as previously described (Mann, 1925). It would thus seem that we had carefully controlled our results to meet the possible objection that the decreased blood flow to the liver, necessary for the complete extirpation of the liver alone, vitiated our conclusions. In those experiments in which only a reverse Eck fistula had been performed, as emphasized in a previous paper, the blood flow through the liver was increased. In the experiments in which the vena cava had been completely or partially ligated, it does not seem as though the liver could have been much affected at all. However, since the pigment always appeared in plasma and fat whenever the liver was removed by any of these methods, it seems advisable to rule out definitely any possible effect of previous operative procedures, by removal of the liver and other intra-abdominal organs in animals which had never been subjected to preliminary operations. We wish to emphasize again, however, that all such experiments are not the same as uncomplicated hepatectomy although the effects of loss of the liver predominate.

In this paper we will present the results of a comparison of the curve of

light transmission of the yellow pigment which developed in the blood of totally dehepatized animal, in the blood of an animal totally dehepatized without preliminary operation, in the blood of an animal with its common bile duct obstructed and gall bladder excised, and with the bilirubin found in the gall bladder of the dehepatized animals at the time of removal of the liver.

METHODS. The method employed for total extirpation of the liver was the same as that described several years ago (Mann and Magath, 1921). All operations were performed under anesthesia and with sterile technic. It should be emphasized that the liver was always wholly removed. As a control to the experiments with the totally dehepatized animal, the common bile duct was ligated and the gall bladder removed in a normal dog, since we have previously shown that following this procedure the bilirubin appears in the blood at a rate comparable to that of the appearance of the yellow pigment in the blood of the totally dehepatized animal (Mann and Bollman, 1925). The method for total extirpation of the liver without preliminary operation will be given with the general results of the procedure in a later paper. It will suffice here to say that the liver and all the other intra-abdominal viscera were removed from a normal dog, care being taken to preserve a sufficient venous return from the lower extremities. At appropriate intervals, specimens of plasma from the various animals were secured and subjected to analysis by the spectrophotometer. In some instances, specimens of bile taken from an animal's gall bladder were compared with specimens of its plasma. Comparative chemical estimates by the van den Bergh method were also made.

The spectrophotometer used in these investigations was of the type recently brought out by Keuffel and Esser, referred to by the manufacturers as a "color analyzer."[1] It consists essentially of a lamp-house carrying two magnesium blocks (cut from the same cake) placed at the rear, which serve as sources of light. The beams of light reflected by these blocks, after passing through two suitable openings in the front of the lamp-house, enter the two receptacles, containing the solution and the solvent, respectively, which are placed in proper position in front of the slit of the spectrophotometer. The spectrometer proper does not differ in fundamentals from the ordinary constant-deviation type of instrument except for a biprism, placed in front of the telescopic lens system, and an observing (exit) slit in the eyepiece. A balance in the intensities of the light transmitted by the standard cell and by the cell containing the solution under examination is made by means of a sector photometer, the intensity of light transmitted by the standard cell only being varied. In all of the

[1] Photographic and cross section drawings showing the optical parts of this apparatus are to be found in an article written by Wallace R. Brode, Journ. Amer. Chem. Soc., 1924, xlvi, 581.

experiments described in this paper, both cells or containers were tubes 1 cm. in length. The standard cell was filled with distilled water, the other carried the unknown solution. This procedure was followed in order to compensate for the effects produced by the end-pieces of glass, which were

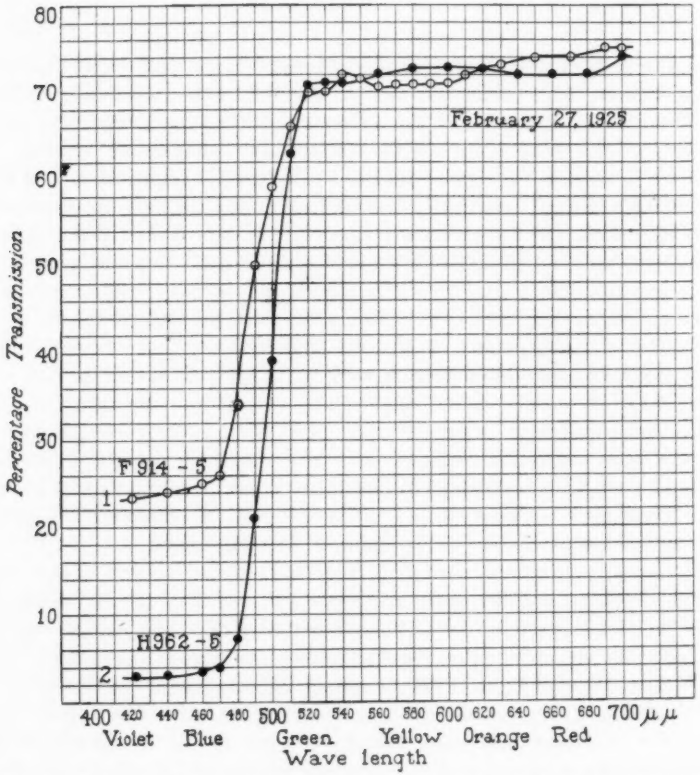

Fig. 1. Curve *1*. Transmission curve, alcoholic extract of plasma obtained 17½ hours after complete removal of the liver. Bilirubin concentration by the van den Bergh method was 1.2 units.

Curve *2*. Transmission curve, alcoholic extract of plasma obtained 18 hours after ligation of the common bile duct and extirpation of the gall bladder. Bilirubin concentration by the van den Bergh method was 2.7 units.

about 2 mm. thick. Otherwise the standard cell need have contained nothing but air.

The method of getting the data shown graphically in the curves consists of taking readings of the intensity of light as it varies with the setting of the wave length (figs. 1, 2, 3). White light is passed through the two con-

tainers, placed in front of the housing containing the rotating sectors, and then admitted to the spectrometer. The spectrometer is set for any desired wave length by means of a calibrated wheel. Let us take, as an illustration, a setting at 640 $\mu\mu$, which is in the red region of the spectrum.

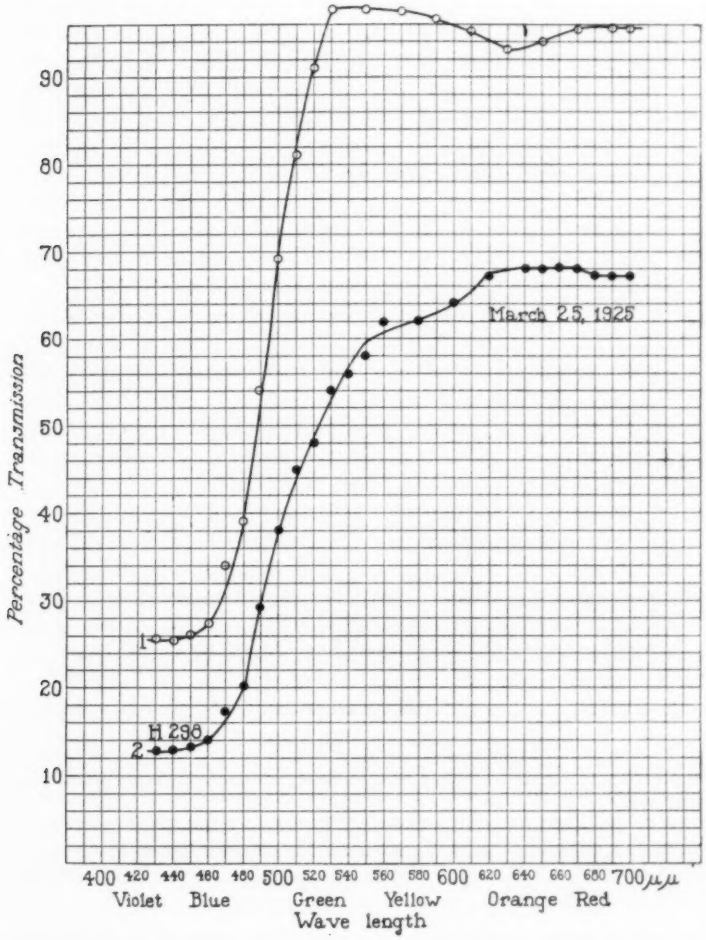

Fig. 2. Curve *1*. Transmission curve, alcoholic extract of plasma obtained 12 hours after complete removal of the liver. Bilirubin concentration by the van den Bergh method was 1.2 units.

Curve *2*. Transmission curve, great dilution of alcoholic extract of bile obtained from the gall bladder of the same animal immediately after operation.

Through the eye-slit one sees two small semicircular colored areas in close juxtaposition, with the dividing line horizontal. Both halves of the circle will have the same color (for example, red) but not necessarily the same brightness or intensity. The adjustment for equality of brightness, or

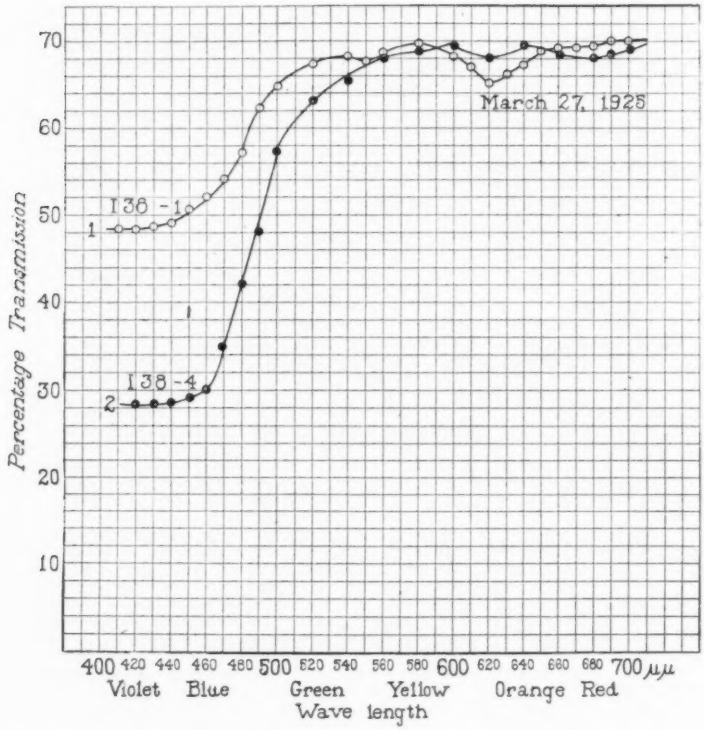

Fig. 3. Curve *1*. Transmission curve, alcoholic extract of normal plasma obtained previous to operation. This was colorless, and tests for bilirubin gave negative reactions.

Curve *2*. Transmission curve, alcoholic extract of plasma obtained 13½ hours after complete removal of the liver and all other abdominal viscera. The animal had no preliminary operations. Bilirubin concentration in the plasma was 0.6 unit by the van den Bergh method.

match, is then made by varying the size of the sector opening in the rotating sectors placed in front of the spectrometer. The percentage transmission of the solution as compared with the percentage transmission of the solvent in the so-called standard cell is read directly from the calibrated drum-head. This drum-head is mechanically attached to the

sectored discs in a manner to permit rapid turning by hand, thus providing a quick way of varying the relative proportions of open and closed sector areas and the amount of light permitted to pass. After the measurement of the equality of brightness for any given wave length has been made, the same procedure is repeated for as many determinations as desired, at any given wave length and for as many spectral regions or wave lengths as the observer wishes. The wave lengths at which readings are made constitute the abscissa; the varying relative amounts of light transmitted by the solution constitute the ordinates.

RESULTS. A yellow pigment appeared in the plasma of each animal. The rate of development of the pigment and the amount as estimated by the van den Bergh method were the same as in the numerous experiments reported previously (Mann, 1925), (Mann, Bollman and Magath, 1924). Plasma obtained from these animals before operation or within two hours after operation was colorless and gave negative results to tests for bile pigment. The spectrophotometer, however, showed a trace of bilirubin even in colorless plasma from the normal dog (fig. 3, curve 1). Within three to six hours after operation, either complete extirpation of the liver, extirpation of all the abdominal viscera, or ligation of the common bile duct with extirpation of the gall bladder, the plasma of these animals became yellow, and positive reactions for bilirubin were obtained by the van den Bergh and other methods. The amount of bilirubin in the blood of these animals progressively increased and bilirubin was found in large amounts in the urine. The rate of development of bilirubin varied with the individual animal, but no striking difference was noted in the three types of experiments described. Comparable amounts of bile pigment developed in the plasma of dogs in which the excretion of bilirubin had been prevented either by extirpation of the liver or by ligation of the common bile duct with excision of the gall bladder.

As determined by the spectrophotometer, the curves of light transmission of the pigment developed in the plasma of the dehepatized dogs; of the animals with all their intra-abdominal viscera removed; of the animals with obstructed common bile duct and gall bladder removed; and of the pigment in the bile obtained from the gall bladder, were identical in nature. The van den Bergh test gave the "direct reaction" with diluted bile from the gall bladder and with plasma containing considerable bilirubin from animals in which the common bile duct had been ligated and the gall bladder removed. The reaction was of the "delayed type" with plasma from animals after extirpation of the liver, but the reaction was accelerated with the increased pigment concentration in these animals, and in certain instances was diphasic in character (Feigl and Quarner, 1919). Curves of light transmission through alcoholic filtrates of these plasmas, however, show that the bilirubin in each is identical.

DISCUSSION. As a means of identifying unknown substances, chemical methods are often advantageously supplemented by spectroscopic examinations. When substances in solution give definite and narrow absorption bands, spectrograms are satisfactory in determining their identity. If, however, as in the case of bilirubin, the essential characteristic is a decrease in the intensity of transmission of certain spectral regions only, then spectrophotometric analysis provides a more sensitive and generally satisfactory method of examination. This is particularly true in investigations such as those described here, for the reason that the concentrations of the various plasmas and other fluids varied so widely as to make it impractical, under the procedure followed, to depend on spectral absorption as demonstrated on the photographic plate or as seen by the eye.

Bilirubin dissolved in alcohol or chloroform shows no sharp absorption bands in the visible spectrum, as has been shown by various investigators, (Bier and Marchlewski, 1902), (Gambee, 1896), (McNee, 1923) but causes decreased transmission for the shorter wave lengths of light. Gamgee and Bier and Marchlewski assert that there is a band at the extreme violet end. All of the curves of figures 1, 2 and 3 shows an approximately uniform transmission from 700 to 540 $\mu\mu$, with a rapid absorption of light from 540 to 460 $\mu\mu$, at which wave length the transmission again becomes practically uniform. All curves give the characteristic absorption zone from about 520 $\mu\mu$ to the end of the scale of the instrument used, that is, 420 $\mu\mu$. No attempt is made here to account for some of the secondary absorption zones, such as that from 600 to 640 $\mu\mu$, for example, in the curves of figure 3, since they presumably have no relation to the object of these experiments.

It may be noted that the percentage of light absorbed in the short end of the visible spectrum varies in the different curves shown in figures 1, 2 and 3. Since we are dealing with the same absorption zone, it is possible to explain these variations in transmission percentages through a determination of the extinction coefficient ϵ, from the general equation $\epsilon = \log \frac{I'}{x}$, where I' is the intensity of the light passing through a layer of thickness d, and x is the depth of the solution used. We have used containing tubes of 1 cm. depth, hence x becomes unity, and therefore $\epsilon = \log I'$. By Beer's law, the absorption of light by solutions is directly proportional to the concentration; hence, knowing the extinction coefficients, ϵ, we can estimate concentrations, c, from the simple relation: $\frac{c_1}{c_2} = \frac{\epsilon_2}{\epsilon_2}$. These facts are presented solely to qualify the statement that the curves of figures 1, 2 and 3 are identical. They are identical in general characteristics, but vary in percentages of absorption in the short wavelength region because of differences of concentration.

Using this formula for the absorption of light by the extracts (alcohol 2 parts) plasma 1 part, of plasma obtained from the animals in these experiments, we find that the relative concentrations of bilirubin are nearly identical with the concentrations determined by the van den Bergh method. We have therefore reported our findings in van den Bergh units. The rate of accumulation of bilirubin in the blood varies considerably in the different dogs of each of our series of experiments. This variation is probably due to the different amounts of fat present in the animals studied, since the fat is always found to have absorbed considerable amounts of bilirubin whenever retention of bilirubin has been produced. We have also observed that the accumulation of bilirubin in the blood of fat dogs is less rapid than of thin dogs similarly treated. For this reason it is difficult to determine the exact quantity of bilirubin formed by dehepatized animals. However, the amount of bilirubin found in the blood of dogs, after ligation of the common bile duct and extirpation of the gall bladder, is so similar to that found in the blood following hepatectomy that the presence of the liver certainly cannot be the predominating factor in the production of bilirubin.

It is interesting to note that the plasma of a normal dog, which is perfectly colorless and gives no other test for bile pigment, contains small amounts of bilirubin detectable with the spectrophotometer. The amount of bilirubin normally found in the plasma of different species of animals is constant for that species. It would appear that the normal threshold of excretion of bile pigment by the liver is higher in some animals than others, but that the liver does maintain a definite threshold for bilirubin.

The final evidence has been secured for proving that bilirubin can be formed without the intervention of the liver or any of the other intra-abdominal organs. The pigment which appears in the urine and blood and accumulates in the fat of the totally dehepatized dog is bilirubin. Its development is not the sequel to any operative procedure other than the total extirpation of the liver. The extra-hepatic formation of bilirubin is an absolutely proved fact. There can be no question but that the liver must be considered, to some extent, as an excretory organ for bilirubin.

SUMMARY

We have previously reported the appearance of a pigment in the urine, plasma and fat of all totally dehepatized dogs, if they lived six hours or more after operation, which gave a positive reaction to the chemical test for bilirubin. We have determined the curve of light transmission of the pigment and compared the curves obtained with the bilirubin in the plasma of a control dog, with the common bile duct obstructed and gall bladder excised, and with the curve produced by bilirubin removed from the gall bladder of the dehepatized animals at the time of operation. The general

character of all the curves was the same. This would seem to be final evidence that the pigment which develops in the dehepatized animal is bilirubin and that extra-hepatic formation of bile pigment is possible.

PROTOCOLS

Experiment 269-24. This experiment was performed for the purpose of spectrophotometric study of the bilirubin appearing in the blood following complete removal of the liver. Other data obtained are eliminated for simplicity.

Dog F914 was an adult female, white and black mongrel in good condition, weighing 11.2 kgm. March 28, 1924, an operation for a reverse Eck fistula was performed. January 7, 1925, the portal vein was ligated. The animal remained in good condition, weighing 10.2 kgm. February 26. On this date at 8:30 a.m. the first specimen of blood was obtained from the jugular vein and the first specimen of urine was obtained by catheterization. At 8:40 a.m. the animal was anesthetized, and the liver was removed at 9:10 a.m. with the usual technic. The operation was completed at 9:30 a.m. and the anesthetic discontinued. The animal weighed 9.7 kgm. following the operation and the liver weighed 250 grams. At 9:55 a.m. the animal had recovered from the anesthesia and appeared normal. Twenty cubic centimeters of 10 per cent glucose solution were injected into the jugular vein. At 11:05 a.m., 12:00 m., 1:05 and 2:10 p.m., the glucose injections were repeated. At 2:55 p.m. the second specimen of blood was removed from the jugular vein, and the second specimen of urine, 60 cc., was obtained by catheterization. Twenty cubic centimeters of a 10 per cent solution of glucose were injected into the jugular vein. Glucose injections were repeated at 4:00, 5:00, 5:50, 7:00 and 8:00 p.m. At 9:00 p.m. the third specimen of blood was withdrawn from the jugular vein and the third specimen of urine, 5 cc., was obtained by catheterization. Twenty cubic centimeters of a 10 per cent solution of glucose were injected into the jugular vein. At 10:00, 11:00, 12:00 p.m., 1:00 and 1:50 a.m. glucose injections were repeated. At 2:50 a.m. the animal died suddenly. The fourth specimen of blood was withdrawn from the heart.

Necropsy (135–25) was performed immediately. The entire liver had been clearly removed and no signs of hemorrhage were present. The fat throughout the body was stained a deep yellow. Nothing was found which would invalidate the results of the experiment.

The plasma from the first specimen of blood was colorless and gave no reaction for bile pigment. Plasma from the second, third and fourth speimens was yellow tinged and contained bilirubin, 0.3, 0.8 and 1.2 units by the van den Bergh method, which gave a delayed reaction increasing in rapidity as the amount of bile pigment increased.

The first specimen of urine gave a negative reaction for bile pigment, but the second and third specimens gave strongly positive reactions.

Plasma from the fourth specimen of blood was precipitated by the addition of two volumes of 95 per cent alcohol. The filtrate of this was examined in the spectrophotometer. The light absorption curves are plotted in figure 1, curve *1*.

Experiment 234-25. This experiment was performed for the purpose of spectrophotometric study of the bilirubin appearing in the blood following ligation of the common bile duct and extirpation of the gall bladder. It is a control experiment for the preceding one. Other data obtained have been eliminated for simplicity.

Dog H962 was an adult male, white mongrel, in good condition, weighing 10.7 kgm. February 26, 1925, at 9:30 a.m. the first specimen of blood was withdrawn from the jugular vein. The animal was anesthetized and the gall bladder removed. The

common bile duct was doubly clamped, severed and ligated at 9:40 a.m. At 9:50 a.m. the operation was completed and the anesthesia discontinued. At 3:40 p.m. the second specimen of blood was withdrawn from the jugular vein, at 5:00 p.m. the third, at 9:40 p.m. the fourth, and at 3:40 a.m. the fifth.

Bilirubin estimations in the plasma in the first specimen of blood were negative; in the second specimen 0.7 unit; in the third, 1.0 unit; in the fourth, 1.8 units, and in the fifth, 2.7 units by the van den Bergh method.

Plasma from the fifth specimen was precipitated with two volumes of 95 per cent alcohol. The filtrate was examined by the spectrophotometer. Light absorption curves are shown in figure 1, curve *2*.

Experiment 630-24. The spectrophotometric examination of the blood of this animal following complete dehepatization and of the bile taken from its gall bladder confirms the belief that the bilirubin found in the blood following hepatectomy is identical with the bilirubin normally excreted in the bile.

Dog H298 was an adult female, black and white mongrel in good condition, weighing 8.9 kgm. June 18, 1924, an operation for reverse Eck fistula was performed with the usual technic. October 15, the animal weighed 8.2 kgm. On this day the portal vein was ligated. March 24, 1925, the animal was in good condition and weighed 8.9 kgm. On this date at 10:00 a.m. the first specimen of blood was removed and the first specimen of urine was obtained by catheterization. At 10:15 a.m. the animal was etherized, and at 10:30 the liver, weighing 200 grams, was removed. The operation was completed at 10:45 a.m. Ten cubic centimeters of 20 per cent glucose solution were injected into the jugular vein at 10:55 a.m., 12:00 m., and 1:10 and 2:15 p.m. At 3:30 p.m. the second specimen of blood was removed from the jugular vein. The second specimen of urine, 50 cc., was obtained by catheterization. Ten cubic centimeters of 20 per cent glucose solution were injected intravenously at 3:35, 4:15, 5:15, 6:00, 7:00, 8:00, 9:00 and 10:00 p.m. At 10:30 p.m. the animal was killed. The third specimen of blood was removed from the heart. The third specimen of urine, 90 cc., was obtained from the animal's cage.

Necropsy (168-25) was performed immediately. The fat throughout the body was stained a dirty yellow. The peritoneal cavity contained a few cubic centimeters of blood-stained fluid. Nothing was found which would invalidate the result of this experiment.

Plasma from the first specimen of blood was colorless and gave no reaction for bile pigment. Plasma from the second and third specimens of blood was yellow. The second specimen contained 0.5 units of bilirubin by the van den Bergh method, and the third 1.2 units. This plasma was precipitated by the addition of two volumes of 95 per cent alcohol and the filtrate examined in the spectrophotometer. Bile from the gall bladder of the animal was diluted with 66 per cent alcohol and examined in the spectrophotometer. The curves of light absorption are shown in figure 2, curves *1* and *2*.

Experiment 327-25. This experiment clearly demonstrates that the preliminary preparation for extirpation of the liver in dogs has no effect on the formation of bile pigment after the extirpation. It also shows that bile pigment may be formed in large amounts without the aid of any abdominal organ.

Dog 138 was a black and white male mongrel in good condition, weighing 10.1 kgm. March 26, 1925, 8:30 a.m. the first specimen of blood was withdrawn from the jugular vein. The animal was etherized at 9:10 a.m. and the operation begun at 9:20 a.m. At 9:30 a.m. the liver was extirpated, and at 9:45 a.m. the stomach and intestines were resected. All the abdominal viscera and both kidneys were removed except a small piece of the pancreas and the two suprarenals. The operation was completed at 9:55 a.m. and the anesthetic discontinued. At 9:55 a.m., 5 cc. of 50

per cent solution of glucose were injected intravenously. At 10:15 a.m. the animal had recovered from the anesthesia and appeared to be in good condition, walking about in its cage. At 11:00 a.m. the second specimen of blood was withdrawn from the jugular vein and 5 cc. of 50 per cent glucose solution were injected. The glucose injections were repeated at 12:00 m., 1:00, 2:00 and 3:00 p.m. At 4:10 p.m. the animal was in good condition when the third specimen of blood was removed from the jugular vein. Glucose injections were repeated at this time, and at 5:00, 6:15, 7:15, 8:15, 9:45 and 10:40 p.m. Five cubic centimeters of 50 per cent solution of glucose were injected intravenously. At 11:00 p.m. the animal was killed and the fourth specimen of blood was taken from the heart.

Necropsy (179-25) was performed immediately. The fat throughout the body was yellow as of bile stain. No signs of hemorrhage were found, and there was nothing present which would in any way invalidate the results of the experiment.

Plasma from the first and second specimens of blood was colorless and gave a negative reaction for bile pigment. Plasma from the third and fourth specimens was yellow, and contained 0.3 and 0.6 units of bilirubin by the van den Bergh method.

Alcoholic extracts (plasma 1 part, alcohol 2 parts) of plasma from the first and fourth specimens of blood were examined in the spectrophotometer. The curves of light absorption are shown in figure 3, curves *1* and *2*.

N. B. Since writing this article a paper on the extrahepatic formation of bilirubin has been published by Rich. He was able to demonstrate the presence of bilirubin in the plasma of dogs in which he had removed all the extra-abdominal viscera, thus corroborating our results that the intra-abdominal organs are not necessary for the formation of bilirubin.

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EFFECTS OF BILATERAL VAGOTOMY ON BLOOD PRESSURE

C. I. REED[1]

From the Hull Physiological Laboratory of the University of Chicago

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In the course of some experiments on the effects of light on blood pressure in the dog (Reed, 1923, 1925) it was noted that sometimes a bilateral vagotomy did not result in the rise in blood pressure commonly described as a characteristic result of that operation. At first it was thought that this was a specific effect of irradiation. However, in some later variations of these experiments, it was noted that the failure of this characteristic response occurred under other conditions. Accordingly, the result of vagisection was recorded in many subsequent experiments. At this time the paper by McDowell (1924) dealing with this phenomenon had not yet appeared.

This author claims that the fall in arterial blood pressure sometimes noted following vagisection is due to the interference with a so-called "vagopressor reflex," set up when pressure in the large veins is very low, thereby originating impulses which pass up the vagi and induce a reflex increase in arterial tone.

It is the purpose of this paper to present the data collected independently, which in certain respects, at least, confirms McDowell's findings.

Blood pressure was recorded from the carotid artery. The vagal trunks were freed and lifted on ligatures so that they could be severed simultaneously with sharp scissors and with a minimum of trauma.

In table 1 are shown the results in 28 experiments of bilateral vagotomy on blood pressure and heart rate when the operation was performed early in the experiments, usually within five minutes after complete surgical anesthesia was induced, and in most cases before any great degree of shock, if any, could be said to have occurred. In the first main column are given the figures representing blood pressure and heart rate before vagotomy. The average blood pressure was 124 mm. Hg, the extreme being 67 and 183; in neither of these cases was there any detectable condition to account for the extremes. The heart rate ranged from 136 to 232. In the second main column are shown the results in detail immediately following vagotomy.

[1] National Research Council Fellow.

In only three instances, experiments 76, 80 and 93, did blood pressure fall after the operation, instead of rising. In two of these the fall was continuous and progressive; in experiment 80 there was a slight subsequent rise after the intial fall but not complete recovery and in a few minutes the depression was resumed. Two of the three cases were below the aver-

TABLE 1

Effects on blood pressure and pulse rate of bilateral vagotomy early in experiments, usually within 5 minutes after complete anesthesia was induced

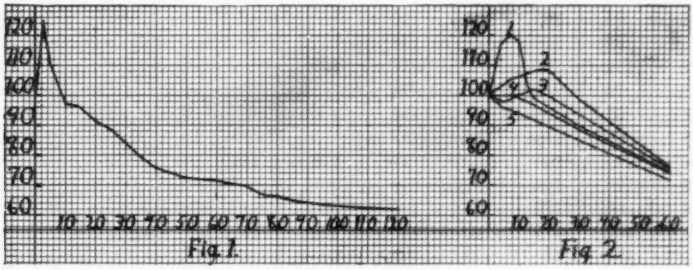
EXPERIMENT	BEFORE VAGOTOMY		IMMEDIATE RESPONSE		5 MINUTES AFTER		LATER EFFECTS	
	Blood pressure	Pulse rate	Blood pressure	Pulse rate	Blood pressure	Pulse rate	Blood pressure	Pulse rate
61	135	142	195	166	134	156		
62	150	208	189	214	146	228		
63	145	204	178	210	159	194	136	172
64	91	140	111	182	108	176	91	172
65	134	156	150	158				
66	103	178	132	186	110	180		
71	113	216	123	200	85	191		
72	134	184	205	192	173	212	163	194
73	94	164	114	168	98	165		
74	147	214	188	242	157	208	146	206
75	140	196	167	186	145	174		
76	117	142	110	158	111	166		
77	134	202	154	204			131	188
78	101	140	124	162			102	152
79	105	148	114		119	154	110	144
80	92	220	73	202	87	188		
81	163	180	182	182	165	162		
82	79	162	82	160	68	156		
89	162	242	178	226	164	180		
90	83	136	88	122	80	102		
91	183	196	199	124	188	200		
92	67	208	90	192	69	232		
93	148	194	145	182	136	182	112	176
94	131	232	181	176	133	192		
95	135	184	158	212	117	202	124	184
97	138	204	200	220	168	180		
98	120	170	144	188	120	162		
99	137	185	160	162	138	158		

age, but there were nine other animals in which the initial pressure was below the average and in which depression did not occur initially, neither was the subsequent fall more rapid than ordinarily occurs.

The third column shows the conditions 5 minutes later and in the fourth column are shown figures at varying periods within 15 minutes, which illustrate the relation between blood pressure and heart rate in later stages.

Further examination of the first two columns of table 1 shows that in two of the three experiments in which there was an immediate fall in blood pressure there was also a progressive decrease in heart rate, and that the subsequent course of the two factors was apparently independent in all three experiments. Moreover, there were eight other experiments in which there was a definite diminution in heart rate following vagotomy, *without* an immediate fall in blood pressure, or an extraordinarily rapid subsequent fall.

The results of early vagisection are further shown in figure 1 which is a composite cuve of a series of 12 experiments extending over a period of two hours in which the animals were subjected to no other experimental procedures. Assuming 100 per cent as the initial level of blood pressure, there was a sharp rise to 124 per cent and an equally sharp fall to the original level within 10 minutes and thereafter a progressive fall totaling 38 per cent at the end of 2 hours.



A classification of the various gradations of response to double vagotomy shows five grades which are illustrated in figure 2, by purely schematic curves drawn to represent the trend of response. The division between the groups of experiments falling into each class is, of course, to a certain extent arbitrary. In class 1 are placed all experiments in which there is a sharp rise of 10 to 15 per cent or more within five minutes after vagotomy. The return was also abrupt as illustrated in figure 1, the further course a progressive but slow fall. In class 2 there was a slow rise of no very great magnitude (3 to 5 per cent), a slow return and a progressive depression of about the same order as in class 1.

In class 3 there was a sharp initial fall (3 to 4 per cent) probably due to traumatic stimulation of depressor fibers, followed by a rise which sometimes did not carry the blood pressure to the initial level, but usually carried it slightly above this point. The return was slow and the subsequent depression similar to that in class 2.

In class 4 there was no change in the blood pressure level for periods varying from 30 seconds to 10 minutes after which there was a depression

TABLE 2

Effects of bilateral vagotomy late in experiment

EXPERIMENT	BEFORE VAGOTOMY		IMMEDIATE RESPONSE		5 MINUTES AFTER		LATER EFFECTS		ORIGINAL PRESSURE
	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	
67	31	160	32	158	33	154	34	150	111
68	60	176	60	174	60				120
69	99	150	110	96	99	180			118
87	36	172	35	168					145
88	35	178	28	172					136
101	81	238	62	236					123
104	69	176	116	146	70	172			144
105	98	200	88	202	94	200			138
106	106	170	131	208	129	208			183
107	84	150	60	160					124
108	100	178	89	184					125
109	113	154	87	154					125
110	91	200	71	170					125
111	62	140	42	154					86
115	129	177	150	177	142				140
116	76	231	62	225					131
117	31	189	22	192					114
118	100	153	91	153					113
119	113	135	152	177	125	159			125
120	78	144	99	195					127
121	68	192	37	198					113
122	102	213	120	231	100	231			148
123	102	216	133	228					125
124	111	255	117	255					129
126	30	132	23	164					136
128	74	208	64	208					143
129	83	192	87	200	54	200			154
130	66	224	63	224					96
131	19	148	18	168					85
132	95	142	102	152					116
133	90	136	55	164					131
135	28	186	31	186					88
136	30	192	26	168					163
137	43	144	36	144	52	106			145
138	144	174	136	198	123	201			158
139	36	165	32	162					89
140	40	144	40	174					94
141	90	138	87	138					148
142	73	183	47	186					87
143	46	128	48	172					174
144	44	147	51	147	45	141			
145	111	93	104	108					111
146	112	180	100	186	120	172			117
147	41	152	37	176					126

TABLE 2—*Concluded*

EXPERIMENT	BEFORE VAGOTOMY		IMMEDIATE RESPONSE		5 MINUTES AFTER		LATER EFFECTS		ORIGINAL PRESSURE
	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	Blood pressure	Pulse	
148	126	156	114	162	120	156			123
149	154	201	174	201					160
151	117	264	142	268					167
153	117	184	158	192					217
160	72	152	74	160	82	228			117
161	40	138	47	141					108
162	70	156	91	180					107
164	120	210	144	216					133
165	56	104	61	112	46	116			136
166	133	198	158	210					157
167	70	152	49	144	61	148			117
168	62	252	47	240					91
169	114	218	98	224					140
170	89	212	70	214					132
172	97	180	103	171	95	180			151
173	94	168	85	180	120	183	107	177	166
174	101	198	97	185					158
175	144	264	151	264	177	273			182
177	97	204	85	222	80	219			125
178	71	144	69	148	78	156	62	201	94
179	76	164	61	160					146
180	85	204	81	213	61	210			103
189	81	200	64	212					118
191	128	224	126	240					114
192	37	228	30	292					127
196	29	162	23	152					81
197	109	198	127	201					136
198	59	140	52	134					104
199	55	168	48	168					163
200	139	210	147		141	216			153
201	115	248	106	243					168

about parallel to that occurring in class 1. In class 5 there was an immediate fall in blood pressure following vagisection, frequently abrupt, evenly progressive, never recovering and likely to reach a lower level than in any of the other classes.

In series I (table 1) all but five experiments fell within class 1 (82 per cent); one in class 2; two in class 3; none in class 4; two in class 5.

In table 2 are shown the results of bilateral vagotomy in 75 experiments in which the animals had been etherized for periods varying from one to three hours and had, in the meantime, been subjected to a variety of conditions which might produce more or less shock. Along with these figures are shown the original blood pressures.

Of the 75 cases, 43 showed a fall in blood pressure as an immediate effect of vagisection, and in all but seven cases the fall was progressive. In eight experiments (105, 137, 146, 148, 167, 173, 178) there was a tendency to recovery after the initial fall, usually only transient, though in experiment 173 the pressure persisted for 20 minutes above the original level, falling quite rapidly thereafter. In 23 cases there was an increase in heart rate as the fall in blood pressure occurred. In the other 20 cases the heart rate was either unchanged or was decreased. In 20 of the 75 experiments, the depression in blood pressure at the time the vagotomy was performed, amounted to less than 20 per cent of the original level. Only 9 of these are included among the 43 cases of blood pressure depressed after vagotomy.

From these figures it is apparent that depression in blood pressure after bilateral vagotomy is not dependent on heart action nor on the previous state of the arterial blood pressure, although in 70 per cent of those animals responding in this manner a fall of more than 20 per cent had actually already occurred. But of the 32 cases in which no fall occurred as an immediate effect of vagotomy, 20, or 62 per cent, had already undergone a depression of more than 20 per cent from the original level of blood pressure.

TABLE 3

	PER CENT
Class 1	16.6
Class 2	20.5
Class 3	7.6
Class 4	11.5
Class 5	43.6

In table 3 are shown the percentages of the experiments in series II (table 2) falling into each of the classes exemplified in figure 2. It is believed that the gradation from class 1 to class 5 represents progressively greater degrees of shock to the animal. However, it is difficult to understand why an animal with a relatively high blood pressure and displaying no noticeable evidences of a pronounced degree of shock will fall into class 5 while another may have a blood pressure of only one-fourth the original magnitude and in addition slow heart rate and small amplitude of beat, and fall in class 1, or, more frequently in class 2. This paradox occurred many times and served to emphasize the partial independence of post-vagotomy depression from a preëxisting condition of low arterial pressure; however, it is apparent that such depression most commonly is superimposed upon an already depressed condition.

In table 4 is shown a classification of the experiments included in series I according to the relation between heart rate and blood pressure.

In table 5 is given a similar classification of series II, together with an analysis of each of the groups with relation to the degree of blood pressure depression present before vagotomy. A figure of 20 per cent reduction from the initial level is taken as a basis of classification.

Of the nine groups of results included in this table only three, including 27 cases, or 36.6 per cent, cover experiments in which there was a characteristic rise in blood pressure following vagotomy. This then leaves 48 experiments, or 63.4 per cent, in which no rise occurred. Among these

TABLE 4

CONDITIONS	NUMBER OF EXPERIMENTS
BP+, HR+	15
BP+, HR−	7
BP_0, HR_0	1
BP−, HR+	1
BP+, HR_0	2
BP−, HR−	2
	28

TABLE 5

CONDITIONS	TOTAL NUMBER OF EXPERIMENTS	20 PER CENT REDUCTION IN BLOOD PRESSURE FROM ORIGINAL LEVEL	MORE THAN 20 PER CENT REDUCTION IN BLOOD PRESSURE FROM ORIGINAL LEVEL
BP+, HR+	13	6	7
BP−, HR+	18	6	12
BP+, HR−	5	1	4
BP_0, HR_0	3	0	3
BP_0, HR−	3	0	3
BP+, HR_0	9	3	6
BP_0, HR+	4	1	3
BP−, HR−	10	0	10
BP−, HR_0	10	3	7
Totals	75	20	55

are 10, or more than 20 per cent, in which the observed results followed a small reduction in blood pressure. All other results, nearly 80 per cent, followed a preliminary reduction in blood pressure of more than 20 per cent, which represents a definite condition of shock.

Thus, while the post-vagotomy fall in blood pressure *may* occur in dogs which, prior to vagotomy, show a normal arterial pressure, it is apparent from these experiments that it is more likely to occur in some condition of shock in which the original blood pressure level has been greatly de-

pressed. However, since these conditions are fulfilled in such a large proportion of cases in which the above-mentioned phenomenon does *not* occur, it is apparent that there is no close correlation between shock and the conditions which McDowell believes responsible for the "vago-pressor reflex," i.e., lowered pressure in the great veins.

The absence of correlation between blood pressure and heart rate changes in these experiments (tables 4 and 5) also confirm McDowell's conclusion that alterations in heart rate are not responsible for the fall. In cases of vagisection in which the fall in arterial pressure does not result, but in which there is also definite evidence of profound shock, it is difficult to apply McDowell's hypothesis for the question at once arises as to the means by which pressure in the great veins is maintained when the general arterial pressure is so low.

In consideration of the question of changes in the volume output from the heart being responsible for the post-vagotomy depression, an inspection of the graphs shows no pronounced nor consistent change in registered pulse pressure as would be the case if this factor were consistently and extensively involved.

These experiments confirm McDowell's findings that the post-vagotomy depression *may* occur when there has been a lowering of general arterial pressure when the latter is well maintained; also that it may occur independently of heart rate or output.

However, the fact that this depression is not a constant sequence of vagisection in conditions of shock as shown in many of these experiments raises the question as to *whether the vagi constitute the main afferent paths for this hypothetical pressor reflex from the great veins.* Is it not possible that in some animals the main pathway may be in the splanchnic trunks? It is difficult on any other assumption to explain the refractoriness of some animals, if the "pressor reflex" actually exists.

It has also been suggested that under conditions of sock when the vagus centers are depressed, cutting the vagi may traumatically stimulate the depressor fibers. This is probably what occurs in those experiments in class 3 (fig. 2). But here again the question arises as to why this same factor does not operate in all experiments when the same technique is employed. Furthermore, under no other condition of experimental stimulation of the vagi does a minimal stimulus induce a persistent and progressive decline in blood pressure such as occurs in class 5; a maximal stimulus would cause a more precipitate fall and in addition would affect the heart rate more consistently than is the case in these experiments.

Another question that must be considered is the possible effect of respiratory alterations resulting from vagisection on blood flow through the lungs and hence on the degree of filling of the heart. But an examination of respiratory changes occurring in these experiments does not reveal

them to be sufficiently constant to give much support to the idea of this phenomenon being a major factor.

Still another possible explanation is to be found in the known relation between the vagus and vasomotor centers. In case of stimulation, or withdrawal of afferent impulses from either center, there may occur either augmentation, depression, or no change in the previous state of tonic activity. This being the case, then section of the vagi may result in either no change, or augmentation, or depression of the tonus of the vasomotor center, depending on whether or not there are impulses passing via the vagi and on the influence that these impulses may be exerting on the vasomotor center, regardless of the site of origin of the impulses. It must, of course, be admitted that no direct evidence is available in these experiments, supporting this view but the results in general suggest such a mechanism.

SUMMARY

1. An analysis is presented of 103 experiments in which the results of bilateral vagotomy were studied.

2. There was no close correlation between cardiac and vasomotor responses to this operation.

3. In experiments in which the operation was performed in the early stages of anesthesia and before any pronounced degree of shock had occurred, the predominant response was a rise in blood pressure with subsequent fall and with varying effects on heart rate.

4. In another series in which the operation was performed in later stages of anesthesia and after varying degrees of shock had been induced, the predominant immediate response was a fall in arterial pressure independent of changes in heart rate, which was persistent and progressive in nearly all cases.

5. In many experiments in which a profound degree of shock had occurred the response to vagisection was a characteristic rise in arterial blood pressure, showing that the post-vagotomy depression in blood pressure does not invariably follow a condition of general shock.

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NUTRITIVE VALUES AND PHYSIOLOGICAL EFFECTS OF DIFFERENT FATS AND OILS

F. J. McCLURE AND R. H. CARR

From Purdue University

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The large number of fats and oils such as margarines, cooking fats and salad dressings which have been widely introduced as foods in recent years makes it desirable to extend greatly our knowledge of each individual fat or oil by more accurate determinations of its component glycerides and fatty acids and also of its special physiological effects when used as a food. The data given by Lewkowitsch (7) as to the per cent of olein, palmitin, stearin, etc., in many of the common fats and oils are quite conflicting and less still is known as to what is a desirable ratio of any one of the above fats to another and what response the animal should make when fed certain fats.

PREVIOUS INVESTIGATIONS. Until recent years the nutritive value of an edible fat or oil has been thought to depend largely upon its co-efficient of digestibility but the examination of a large number of fats and oils by Langworthy and Holmes, Deuel (1) and others, has shown that they vary but slightly in this respect, the majority being quite thoroughly assimilated. They reported that subjects eating 100 grams per day of cocoa butter as a part of the regular test meal, experienced disturbances characterized as "loss of appetite," "headache" and "nausea" and the ration proved generally laxative. In the same experiment subjects eating 140 grams of rendered beef suet reported a laxative effect not produced by butter, lard or mutton fat. Goose fat proved laxative, while chicken fat was not laxative. The nutritive values of stearic, palmitic, myristic, lauric, capric and oleic acids have been determined by feeding experiments with young rats. The acids of lower molecular weight were found to be better; and oleic acid was better than stearic acid (2).

Since the early experiments of Eijkman, pigeons have been used extensively for the study of the anti-neuritic vitamine. Small quantities of fats and lipoids were fed to mature pigeons by Takahaski (3) in an endeavor to ascertain the vitamin B content of such substances. Sugiura and Benedict (4) have prepared synthetic rations containing as high as 30 per cent of butter fat in one instance, and have been successful in feeding the ration to pigeons confined under laboratory conditions. A ration com-

prised solely of whole wheat grains plus a mineral supplement such as was used as a basal ration in this experiment has been found adequate for maintenance and growth of pigeons (5). Analyses of feces from pigeons receiving a ration of soybeans plus a mineral supplement and another of sunflower seed plus a mineral supplement, showed the large amounts of fat in these two cereals to be digested quite thoroughly by the pigeons.

OUTLINE OF EXPERIMENT. After accounting for all factors necessary to a complete ration, it appears possible that a particular fat or oil administered with the ration might become the outstanding cause of differences in the physiological responses of mature pigeons. It was thought that different effects would be indicated in more than one of the following ways: *a*, the general behavior and response of the birds, indicated largely by their body weight taken at ten-day intervals; *b*, the daily consumption of the basal ration of whole wheat grains; *c*, the physical state and the amount of the crude fat of the feces; *d*, the number of eggs produced, the amount of fat in the eggs and the variations in certain constants of the resulting egg fat.

The birds for the experiment, secured from a nearby farm, were dusted with sodium fluoride and then placed in a warm, well-ventilated room in good light. Those which showed evidence of lack of vigor were discarded and only the most healthy retained and mated. They were placed on a ration of whole wheat grains (Michikoff variety) and supplied with grit, oyster shell, ground bone, salt, charcoal and tap water. They were kept on this ration until eggs were produced and proved to be fertile, and then the feeding of the fats and oils was begun. Commercial fats and oils or their products, melted if necessary and filtered to free them from other substances, were given to the birds by forced feeding with a syringe, graduated in cubic centimeters. Each pen containing a pair of birds was provided with a small bottle in which the fats or oils for about one week's feeding were kept. The fats were warmed to the liquid state in a hot water bath just previous to the feeding, and each morning the birds were removed from the cages and the syringe, filled with the desired amount of fat or oil, was injected well down in the pigeon's throat and quickly emptied. Stock supplies of fats and oils were kept in tightly stoppered bottles at 0°C. The wheat fed contained 12.9 per cent crude protein (air dried basis) and 2.1 per cent crude fat. The birds ate an average of 18 grams of wheat per day per bird, during the second period when 3 cc. of the "fat substances" were fed every other day. Their ration contained thus about 8.8 per cent of fat counting 2.1 per cent fat contained in the wheat. In the third period, when 5 cc. of the fat or oil were fed every day, the birds consumed on the average 10 grams of wheat per day per bird. The amount of fat during this period was about 32 per cent of the total ration.

Previous to feeding the quantities of fat shown in figures 1 and 2 the

birds had been fed 2 cc. of oil every other day for about one month. At the end of this period each pair of pigeons was changed to a different kind

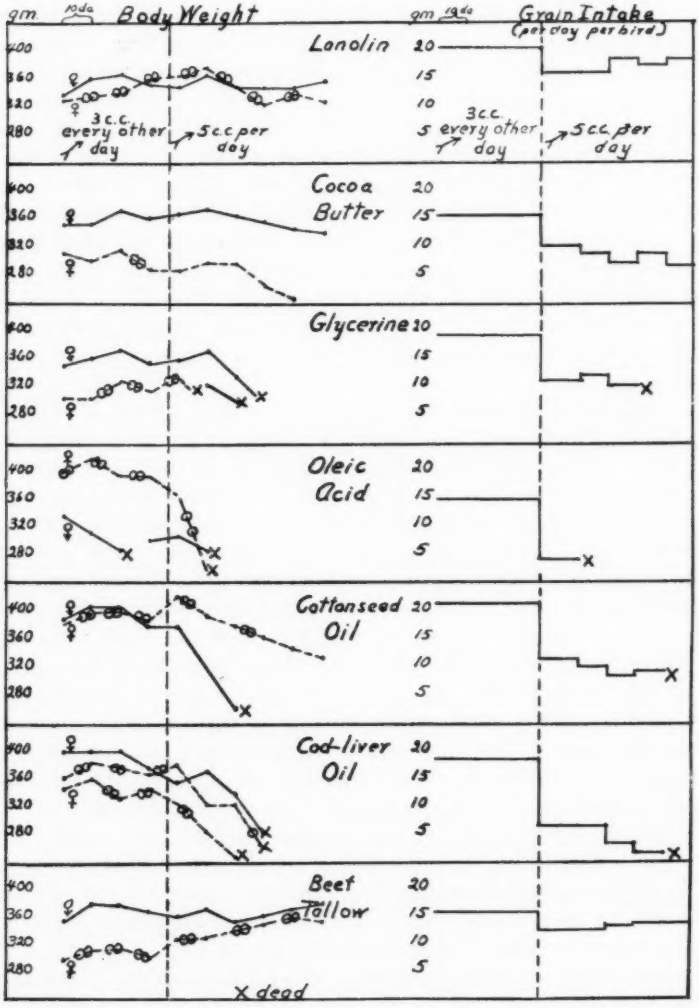

Fig. 1. Body weight, grain and fat intake and egg production during second and third feeding periods.

of fat for the second feeding period. Nothing characteristic of the fat developed during this first period. The per cents of crude fat in the feces

as shown in table 1 are calculated on a moisture- and ash-free basis and represent the amount of crude fat in the feces above that found in the

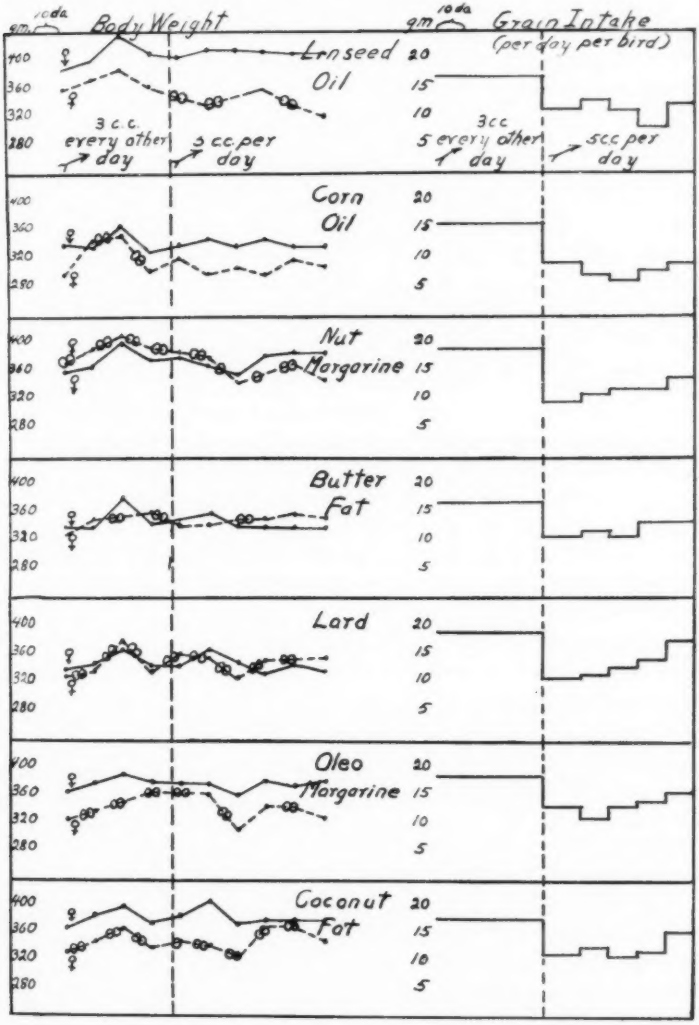

Fig. 2. Body weight, grain and fat intake, and egg production during second and third feeding periods.

feces produced from the straight wheat ration. This latter amount averaged about 2.5 per cent for all pairs.

The acid digestion method was used for the determination of the crude fat in the air dried and finely powdered feces. This method was essentially that recommended by Hertwig for flours and noodles (6). The amount of fat in the feces was generally in agreement with the accepted co-efficients of digestibility except in cases where serious toxicity developed. Differences in the daily consumption of whole wheat grains became particularly significant during the feeding of 5 cc. of fat compounds every day. In those cases where the decline in weight was most rapid, little wheat was eaten, as would be expected. In the case of birds receiving lanolin, beef tallow and cocoa butter, the consumption of wheat was high because of the relatively low digestibility of the fat or oil. On the other hand birds receiving cocoanut fat continued to eat relatively large amounts of wheat despite the fact that there was but little waste of fat indicated by the feces.

Cocoa butter contains an unusually high amount of stearic acid (about 40 per cent) together with palmitic and arachidic acids and about 31 per cent of oleic (7). The birds receiving this fat could hardly be considered

TABLE 1

Fat content (in per cent) of feces from pigeons fed different oils

	LANOLIN	BEEF TALLOW	COCOA BUTTER	OLEIC ACID	LARD	BUTTER FAT	LINSEED OIL	COD LIVER OIL	COTTONSEED OIL	COCOANUT FAT	GLYCERINE	OLEO MARGARINE	CORN OIL	NUT MARGARINE
3 cc. every other day	15.3	9.8	8.3	3.5	3.3	3.3	3.2	2.8	2.6	2.5	2.0	2.0	1.3	1.2
5 cc. every day	22.7	17.1	17.9	22.6	12.9	12.1	8.1	14.3	13.5	5.4	2.1	10.3	9.1	7.8

normal from the start of the feeding of this substance. The female produced but two eggs and gradually lost weight. It was thought that this female was naturally sluggish and therefore failed to give a reliable response to this fat. Three birds receiving glycerine died rather suddenly after exhibiting for a day or two previous a peculiar nervousness, trembling and leg weakness. The feces indicated extreme laxative effect. Several interesting effects followed the feeding of oleic acid. It was early noted that these birds had developed a very noticeable gloss on their feathers, in striking contrast with the birds which were receiving glycerine and which were noticeably dull in their coat. Nut margarine, oleo-margarine and butter fat produced glossy coats also but to a lesser degree than oleic acid. It is thought that a fat whose glycerides yield large amounts of oleic acid, may cause this effect, but birds receiving corn oil, which contains glycerides of oleic acid (45.4 per cent), linoleic acid (40.9 per cent), palmitic acid (7.7) per cent) along with lesser amounts of several others (8), did not appear unusual in this respect. A severe pulmonary trouble which usually

resulted in death, as well as extreme laxativeness, developed in birds fed oleic acid. Before they died they showed great distress and difficulty in breathing and on autopsy blood clots were found in the throat and lung tissue. Stearic and palmitic acids are reported as causing a like trouble in rats (2). Cottonseed oil, containing mostly glycerides of liquid fatty acids, consisting approximately of 60 per cent linoleic and 40 per cent of oleic acid (the solid fatty acids vary from about 20 to 24 per cents) (7) caused the birds to regurgitate large quantities of wheat and oil. It was also laxative. Cod-liver oil was extremely laxative and toxic in larger amounts. Glycerides of liquid fatty acids compose 85 to 90 per cent of this oil. Some of these acids have the general formula, $C_n H_{2n-8}O_2$ and $C_n H_{2n-6}O_2$, the latter however differing from linolenic acid (7). The birds fed linseed oil, whose glycerides yield about 77.5 per cent linoleic acid and 15.5 per cent linolenic acid (7), remained quite normal; the oil was not laxative and was quite completely absorbed. The birds receiving beef tallow, (several samples of beef tallow were found by Lewkowitsch to contain 21 to 22 per cent stearic acid)[1] produced feces which were dry and white, indicating no laxative effect. It is thought that oleo-margarine possessed some laxative properties. Butter fat did not appear to be laxative nor did it show superior nutritive properties. Glycerine, oleic acid, cottonseed oil and cod-liver proved fatal in large amounts, and cocoa butter gave indications of toxic properties, but it may be said in general that aside from the differences mentioned, the majority of the fats and oils fed interfered but little with the normal functioning of the birds.

The egg production of practically all the normal birds seems to be somewhat above the average, though little is known as to how often pigeons should be expected to lay, when confined under laboratory conditions and not allowed to hatch the eggs. Suguria and Benedict (4) state that on normal diet one pigeon laid four eggs in 34 days, another two eggs. During 147 days on a synthetic diet two pigeons produced 18 eggs. The pigeons used in this experiment averaged one egg every eight days. Aside from those cases where the feed proved seriously toxic, it is concluded that the fat or oil had nothing to do with the number of eggs produced, this function being inherent entirely within the individuals. The eggs produced were tested for fertility in the incubator during all the feeding periods and in practically all instances were found to be fertile.

Crude fat determinations were made on the eggs on the total edible portion, in the liquid state because it was found practically impossible to separate the yolks and whites in such small eggs. The method used followed closely the Röse-Gottlieb method for fat in milk and it is essentially the method recommended by Kühl (9) for the extraction of fat from the

[1] Leathes: *The fats*, p. 80, "beef tallow contains 51 per cent stearic acid."

fluid egg yolks. The edible portion was transferred to a tared glass-stoppered Erlenmeyer flask and weighed. After a thorough mixing of the egg, 2 cc. of concentrated ammonium hydroxide and 5 cc. of 95 per cent ethyl alcohol were added and mixed well after each addition. Twenty-five cubic centimeter portions of ethyl ether and petroleum ether were then used to extract the mass, which was quite jelly-like in nature, and permitted easy decanting. The combined extracts were evaporated to constant weight in a small fat flask in a vacuum desiccator, eight to ten days being required to reach constant weight. The saponification value and iodine number (the Hanus method) were determined as directed in the A. O. A. C. and were made directly on the 0.5 to 1.0 gram sample of fat contained in the original fat flask.

The amount of fat in the total edible portion of the pigeon eggs varied from 4.00 per cent in the case of birds receiving cocoanut fat, to 6.75 per cent in case of birds receiving beef tallow. The average for all birds receiving a fat or oil was 4.96 per cent, while eggs produced during the feeding of the straight wheat ration contained 4.81 per cent fat in the total edible portion. The amount of fat in the whole uncooked hen egg is about 10.5 per cent of the edible portion. This difference between the hen egg and the pigeon egg is partly accounted for by the fact that there is a rather wide difference in the proportion of white to yolk in the two eggs. The hen egg averages 56.2 per cent albumin, 31.7 per cent yolk and 12.1 per cent shell (10), while the pigeon egg is 69.0 per cent albumin, 19.5 per cent yolk and 11.38 per cent shell. The fat in the hen yolk is about 30 per cent; in the pigeon yolk about 22.4 per cent, according to determinations made directly on the pigeon yolk and by calculations based on the above data. So far as is known, there are no data available regarding the fat content of the pigeon egg. The average saponification number of pigeon egg oil from eggs produced during the feeding of the fat compounds was 176, varying from 166 to 190; the iodine number averaged 70.5 with but slight variations from the average. On the whole wheat ration the saponification number averaged 173, and the iodine number 70.8. Griffith (11) reports hen egg oil as having a saponification value of 184 to 190, and an iodine value of 68.5 to 81.6. Lewkowitsch (7) reports the iodine value as determined by Kitt 72.1. Pigeon egg oil resembles hen egg oil in appearance and tends to separate into a solid and a liquid portion at ordinary temperature.

General discussion. The toxic effects produced by certain fats and oils fed to mature pigeons, so far as could be told, had no relation to any of the fat constants, but were more or less specific for a particular fat or oil. Fats with high melting points, as beef tallow and cocoa butter, were not as completely digested, probably because of their compositions and physical state. Lanolin was very poorly absorbed because of the large

amount of unsaponifiable matter which it contains. Linseed oil, a highly unsaturated oil with drying properties produced no harmful results; cod-liver oil also highly unsaturated but without drying properties, due to the different kind of glycerides present, proved fatal in three instances. Cod-liver oil was very laxative and nauseating because of its taste and odor perhaps, though it might be expected that the same thing would be true of linseed oil. Cottonseed oil acted more like cod-liver oil, though it is more closely related to linseed oil in chemical composition. It may be possible that the substance gossypol was active in this instance, though this is not known for certain. It is to be noted that beef tallow did not prove at all laxative to pigeons, which is not in agreement with the results obtained by Langworthy and Holmes (1). The peculiar effect produced by glycerine may have been due to its peculiar hydroscopic properties and to its laxative properties. Oleic acid is known to be laxative but the reason for the pulmonary trouble produced by this substance is not understood.

It occurred to the writers that, as the experiment developed, the results could be explained on the basis of the percents of the fatty acids making up the various glycerides of the fats and oils fed. The difficulty of thus interpreting the results lies in the fact that the analyses of many of the fats and oils in terms of fatty acids are not altogether reliable, because of the conflicting results obtained in so many instances by the various workers. It is recognized also that in most instances no outstanding glyceride predominates in a fat or oil and also that the effects of substances present in small amount (not including vitamines) may seriously affect its nutritive value.

SUMMARY

1. Cod-liver oil, cottonseed oil, oleic acid and glycerine proved fatal to mature pigeons when fed in amounts of 5 cc. every day. Cocoa butter gave indications of digestive disturbances.

2. Oleic acid caused an unusual gloss on pigeons' feathers. Nut-margarine, oleo-margarine and butter fat had the same effect to a lesser degree.

3. The kind of fat in the ration had no effect on the amount of fat in the pigeon egg, nor did it influence the composition of the egg fat so far as the saponification value and iodine value indicate.

4. The best egg production was one egg every five days and the average for all birds was one egg every eight days. The average fat content of the pigeon egg was found to be 4.96 per cent of the total edible portion. The saponification number and iodine number of pigeon egg oil were found to be 176 and 70.5 respectively.

5. Mature pigeons are able to tolerate rather large amounts of different fats when added to a basal ration of whole wheat grains plus a mineral

supplement. It is believed therefore that they are capable of indicating the important physiological properties of different fats.

6. The desirability of the fats tested as foods seems to depend on the specific properties of the fats themselves which are not indicated by any of the known fat constants.

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THE INTRAVASCULAR USE OF HEPARIN

C. I. REED[1]

From the Hull Physiological Laboratory of the University of Chicago

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In the course of investigations on the physiological effects of light (Reed, 1923, 1925) in which a quartz tube with cannulas attached was interposed in the carotid artery, it was found necessary to emply an anticoagulant intravascularly. Hirudin was not available and after unsatisfactory results with nucleic acid, urea and anti-coagulant material described by Mills (1922, 1923) and his co-workers, heparin was employed (1918, 1922). Howell (1925) states that this substance may be injected without injurious effect and the results herein reported confirm this conclusion, so far as our experiments indicate.[2]

However, it may prove useful to other workers to give some detailed results. Heparin is readily soluble in water or salt solutions. For present purposes Ringer solution warmed to about 30°C. was employed. Concentrations varied somewhat, the highest employed being 10 mgm. per cubic centimeter. It was not determined at what point saturation occurred. Solutions have remained stable up to five hours at laboratory temperature.

Coagulation time was first determined on a sample of blood drawn from the femoral artery. This was found to vary greatly, depending on a number of conditions. In one animal repeated determinations showed a normal coagulation time of 2 minutes, though in most animals the time varied from 8 to 10 minutes.

The efficiency of the heparin solution was tested in several cases by mixing a quantity sufficient to contain 1 mgm. with 1 cc. of blood and observing coagulation time. In samples with normally low coagulability, frequently no coagulation occurred at all, the samples finally laking and decomposing. In general it could be said that coagulation time was increased from 100 to 500-fold in experiments in vitro.

Injection was made quickly, usually in the femoral vein. Samples were drawn at intervals thereafter to determine coagulation time. Systemically, injections produced no detectable effects on blood pressure

[1] National Research Council Fellow.

[2] A part of this material was supplied through courtesy of the manufacturers, Hynson, Westcott & Dunning, by arrangements with W. H. Howell.

heart rate, respiration, sensory threshold or any physical or chemical property of the blood, so far as could be determined, except coagulation time. It is therefore an ideal preparation for use in experiments of this nature.

Summarized results of several experiments follow, and will give a clear idea of the results obtained.

Experiment 165. Seven kilo dog. Normal clotting time, 2 minutes; in vitro mixture never clotted. Ten milligrams heparin injected; third sample 5 minutes after injection, clotting time 25 minutes; fourth sample 1 hour after injection; clotting time 8 minutes.

Experiment 173. Thirteen kilo dog. Normal clotting time, 2 minutes; in vitro mixture 270 minutes; 50 milligrams heparin injected; 6 minutes after injection, clotting time 8 minutes; 18 minutes after injection, clotting time 240 minutes; 60 minutes after, clotting time 25 minutes; 100 minutes after, clotting time 5 minutes.

Experiment 188. Fourteen kilo dog. Normal clotting time, 9 minutes; in vitro mixture never clotted; 100 milligrams heparin injected; 6 minutes after sample never clotted; 35 minutes after, clotting time 111 minutes; 45 minutes clotting time 94 minutes; 65 minutes after, clotting time 50 minutes; 105 minutes after, clotting time 15 minutes; 165 minutes after, clotting time 5 minutes; 180 minutes after, clotting time 5 minutes; 205 minutes after, clotting time 5 minutes; 230 minutes after, clotting time 3 minutes; 240 minutes after, clotting time 1.5 minutes; 250 minutes after, clotting time 1.5 minutes; 280 minutes after, clotting time 40 seconds.

From these results it is apparent that coagulation time can be greatly increased. Within periods of an hour, ether anesthesia does not greatly reduce coagulation time. The results of irradiation by any of the various methods already reported (Reed, 1923, 1925) may be illustrated by the following tabulation of coagulation time of samples obtained at intervals shown.

Experiment 185. 9:37, 8 minutes; 9:53, 9 minutes; 10:02, 4 minutes; 10:39, 3.5 minutes; 11:02, 3 minutes; 11:13, 2 minutes.

In all experiments in which heparin was not employed, irradiation caused a progressive decrease in coagulation time. With heparin injection just prior to the beginning of irradiation, the coagulation time decreased at about the same rate but never reached a level of coagulability at which the blood would coagulate in the arterial tube if the dose of heparin was large enough.

Heparin has been employed in a total of 30 experiments, four of which were controls. It has now been determined that a dosage of 10 mgm. of heparin per kilogram of body weight is the minimum dependable dose, although in some instances much smaller doses have been successfully employed. The efficacy of a given dose does not appear to be correlated with the normal coagulability alone but upon some undetermined factor also. Nor has it been possible to correlate the duration of effects with any observed factor.

It was found incidentally that heparin is an excellent anti-coagulant agent for use in samples of blood for chemical analysis. In these experiments such samples were routinely taken for chemical determinations, results of which will be reported later. It was found that defibrination, citration or oxalation interfered with certain determinations while heparin did not.

In samples drawn in test tubes and allowed to stand the cells began to settle out in a few minutes and frequently within a half hour there was as complete separation as is ordinarily obtained by centrifugation. The buffy coat was quite marked in most samples. These tubes could be shaken gently and allowed to stand with the result that sedimentation usually occurred even more rapidly.

Dilution of a blood-heparin mixture with salt solution or distilled water usually induced speedy coagulation.

Clots which finally formed in heparinized blood were seldom as firm as those found in normal blood and they did not usually retract as a normal clot tends to do.

Coagulation may be induced in a blood-heparin mixture by sufficient agitation of the container or even by frequent pouring from one vessel to another. As a precaution, then, it is best, even where the concentration of heparin is high, to avoid pouring or any other extensive agitation. Under these conditions coagulation does not occur immediately but the latent period is likely to be materially shortened.

SUMMARY

1. Heparin may be successfully employed intravascularly without complicating experimental results, so far as indicated by our experiments.

2. Heparin injected is either destroyed or neutralized so that the normal coagulation time is restored in from one to two hours. A second injection will again increase coagulation time without undesirable effects on the animal.

3. Ten milligrams per kilo of body weight is the minimum dependable dosage.

4. Heparin is valuable as an in vitro anti-coagulant where it is desirable to retain all other physical and chemical properties undisturbed.

5. Precautions for its use are mentioned.

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TRAINING THE HEART BY SYSTEMATICALLY REGULATING THE RESPIRATIONS

P. F. SWINDLE

From the Department of Physiology, Marquette University School of Medicine, Milwaukee, Wisconsin

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When the respiratory rate is to the heart rate as 1 is to 2, every second heart beat may be unusually weak while the remaining beats are unusually strong. A graphic record of such a series of beats consists of alternately large and small waves (fig. 2). A series of this sort is sometimes referred to as an "apparent" *pulsus alternans*. However, if the alternately strong and weak beats continue to exist after the respirations are suspended (fig. 4) the phenomenon should be called a "genuine" *pulsus alternans*, at least during the period of apnea.

Knoll (1878) observed the apparent alternans and correctly interpreted it as the result of the action of the respirations upon the circulation. He found that by properly regulating the respiratory rate apparent pulsus alternans and other varieties of grouped beats could be produced. Aguilar (1921) attributed the phenomenon to the aspiration of blood in the pulmonary capillaries during inspiration. Trotter, Edson and Gesell (1922) also attributed the apparent alternans to the respirations. Hirschfelder (1918) looked upon the weaker of the heart beats as dicrotic waves and accordingly spoke of dicrotism instead of an alternans. It will be considered in the present paper that Knoll, Aguilar and Trotter, Edson and Gesell were correct in their judgment that the respirations cause the apparent alternans. It will also be considered that the respiratory phases mechanically affect the ventricular muscle as well as the intrathoracic arteries. Although mild increases in the negative pressure in the thorax may not cause the ventricles to inflate (Wiggers, 1923), direct observation shows that moderately strong inspirations of an animal may not only inflate the ventricles but that, if properly timed, they may also mechanically reduce the extent of ventricular systole. When the natural inspirations are exceptionally strong the ventricles may contract a little at and near the apex while the basal region of the ventricles is greatly distended. In one animal the apex was observed to retreat completely into the heart, presumably into the chamber of the left ventricle, at each strong inspiration.

Frequently associated with both the apparent and the genuine alternans are some periodic variations, slow waves and nodes of a certain type, in the arterial blood pressure as well as in other graphic records of the heart's action (figs. 2 and 5). The nodes mean in some instances a rise and in others a fall in the mean blood pressure. They are usually the troughs of the slow waves. Each of the nodes means a *transition* in the sense that here the beats are of equal strength pending a complete reversal of the preceding relationships with respect to strength of beat; beats which were weak become stronger as those which were strong become weaker, and the point at which such a transition takes place is a node. The transitions often occur at different rates, some quite suddenly and others very slowly (fig. 5). Hirschfelder's tracing shows typical, slow transitions, but he apparently overlooked the fact. Trotter, Edson and Gesell appropriately named the slow, periodic variations in the apparent alternans "cardio-respiratory interference waves," but this name is of course not applicable to the slow variations of similar appearance in the genuine alternans.

The nodes occur in the apparent alternans when the respiration-heart ratio is not exactly 1:2 but is 1:2 plus or minus a fraction. The fraction necessitates that the respirations and heart beats are periodically in and out of step. The respirations either gain or lose the space of a heart beat at regular intervals. The transitions in the genuine alternans are not so easily explained because they occur not only when the heart is intact and the respirations suspended but also when the heart is excised (Swindle, 1922). Mines (1913) attempted to explain the genuine alternans on the basis of a seemingly simple assumption, but his theory does not appear to take care of the transition and must be considered, therefore, as not explaining the alternans. It may be useful to mention here that these transitions are like some in records of simple movements of bodily members of birds and lizards (Swindle, 1915) as well as the human (Swindle, 1916).

In the present series of experiments advantage was taken of the fact that desired patterns of heart beats can be obtained in the experimental animals by properly timing the respirations. Systematic regulation of the respiratory rate is considered a convenient method of *training* the heart to beat in definite, unusual ways. It was hoped that useful information would be gained concerning the relationships between apparent and real alternans. The methods of procedure are important enough that a detailed account of them will be given.

Methods. A dog was anesthetized with ether and arrangements were made to record respirations and heart beats, to observe the heart directly and to mechanically increase and decrease the negative pressure in the chest.

The respirations and heart beats were recorded by various means, some of which will be explained in connection with the tracings presented.

It appeared advisable to record these reactions in several different ways so that the shortcomings of any one method would not stand in the way of interpreting the results. One or more of these methods were used with each animal.

While the dog was given artificial respiration by forcing air through the trachea into the lungs, some skin, flesh and segments of ribs were removed from one side of the chest. The opening made was circular and 9 or 10 cm. in diameter. Bleeding was stopped by ligaturing appropriate vessels. A funnel of very clear glass was fitted underneath the edges of the cut skin and over the opening in the chest wall. The small end of the funnel was connected to a suction pump so that the intrathoracic air pressure could be increased or decreased as desired. This is only a modified form of the usual means of altering the negative pressure in the chest to cause the lungs to inflate and deflate as in natural respiration. The heart could be observed directly through the glass of the funnel. In special cases the direct observations of the heart, diaphragm, etc., were made through a special window of clear, thin glass.

After the operative work was completed, respiration via the trachea was stopped and the normal negative pressure was created in the chest by means of the suction pump. The dog could then breathe of its own accord, and the respiratory rate could be altered either by changing the concentration of the ether vapor in the inhaled air or by mechanically changing the intrathoracic air pressure. As a rule, animals subjected to this treatment breathed more rapidly when the anesthesia was light or when the lungs were in a state of relative deflation than when the anesthesia was profound or the lungs greatly inflated. Apnea could be produced in some of the animals either by increasing and decreasing the negative pressure in the chest rapidly or by holding the lungs in a state of abnormal distension. The vago-sympathetic nerves were always intact in these experiments, and apnea was produced in no case by stimulating vagus nerve elements.

Figure 1 shows clearly one of the methods employed to "train" the heart to execute alternately weak and strong beats during periods of apnea. In this particular instance the respirations were recorded by means of a Marey tambour connected with a rubber tubing to a side tube of the ether bottle. Each time the air was drawn out of the chest with the suction pump, the lungs inflated as air rushed into them through the trachea. Apnea sometimes resulted from thirty to forty minutes after the beginning of artificial respiration which was sufficiently rapid to maintain the respiration-heart ratio of 1:2. Apnea could be caused in this way in only about 30 per cent of the dogs, and two hours or more of the rapid respiration was required to produce apnea in a few of these. At intervals the normal negative pressure was restored in the chest, and

the heart action was observed in order to determine whether the training had been effective. If there was no alternans at any of these times the training was continued after a short interval, as shown in the tracing. In some instances the trouble was not taken to restore the normal negative pressure between periods of training. Instead, the heart action was observed while the lungs were deflated. The amount of training required to cause the hearts of the different dogs to show the alternans type of beat during longer or shorter respiratory rests varied greatly, from a few minutes to over three hours, and in a few instances it was necessary to admit failure. This training method was applied to eighteen dogs. Eight responded well, six poorly and four not at all to the training.

The essential points of the second training method are indicated in figure 2. This method consisted in regulating the concentration of the ether vapor in the inhaled air so that the respiration-heart ratio was approximately 1:2 often for two hours or even longer. It was not possible to maintain the exact ratio of 1:2 very long at a time. Transitions occurred in the apparent alternans in spite of all efforts to prevent them. As soon as each cardio-respiratory interference wave meant a considerable change in the mean blood pressure (the theory back of this is rather vague and will not be stated here), temporary apnea was caused by suddenly distending the lungs and holding them in this state for several seconds. If no alternans appeared during the apnea, the normal negative pressure was again created in the chest and the dog permitted to breathe rapidly for several minutes more. In some cases the concentration of the ether vapor was increased quickly, and indications of an alternans superimposed on the large respiratory variations in the arterial pressure were looked for. If there was no alternans, the concentration of the ether vapor was diminished so that the dog breathed rapidly again. The second training method was applied to more than fifty dogs. About 70 per cent of the animals which could be induced to breathe rapidly enough to cause the pseudo-alternans, reacted favorably to this form of training.

The first and second training methods were modified for use in some experiments to determine whether such results as those in figures 3, 4 and 5 can be attributed to the systematic training or possibly to injured or weakened hearts. The respiratory rate was varied as frequently as possible so that the respiration-heart ratio changed constantly over a long period of time, and the tracings obtained during apnea were scrutinized for indications of an alternans or any other persisting effect. Ten dogs were used for this general purpose. In three of these cases the hearts were badly mistreated with the intention of weakening them without subjecting them to a systematic training. By means of the suction pump the lungs and heart were drawn or even jerked out of the chest and often held in this position for several seconds.

In almost all of the experiments the hearts were observed directly to determine the effects of the respiratory phases on the ventricles and to determine whether the sequence of the auricular and ventricular systoles was normal. In some special experiments two points of the right or left ventricular wall were connected with a thread so that the amount of distortion could be observed at inspiration and expiration. In some instances very small, young leeches (obtained from the ventral side of a mother leech) were placed on the heart, and the amount of distension of these at inspiration was determined with the aid of a dissecting microscope. In other cases only a small circular opening was cut in the pericardium over the right or left ventricle, and observations were made to determine the amount of increase of the negative pressure required to cause the muscle to bulge out at the opening.

Cardiometers were found to be unreliable in one or more respects; if they did not pump air or impede the circulation, they failed for some other reason to indicate reliably the volume changes of the ventricles.

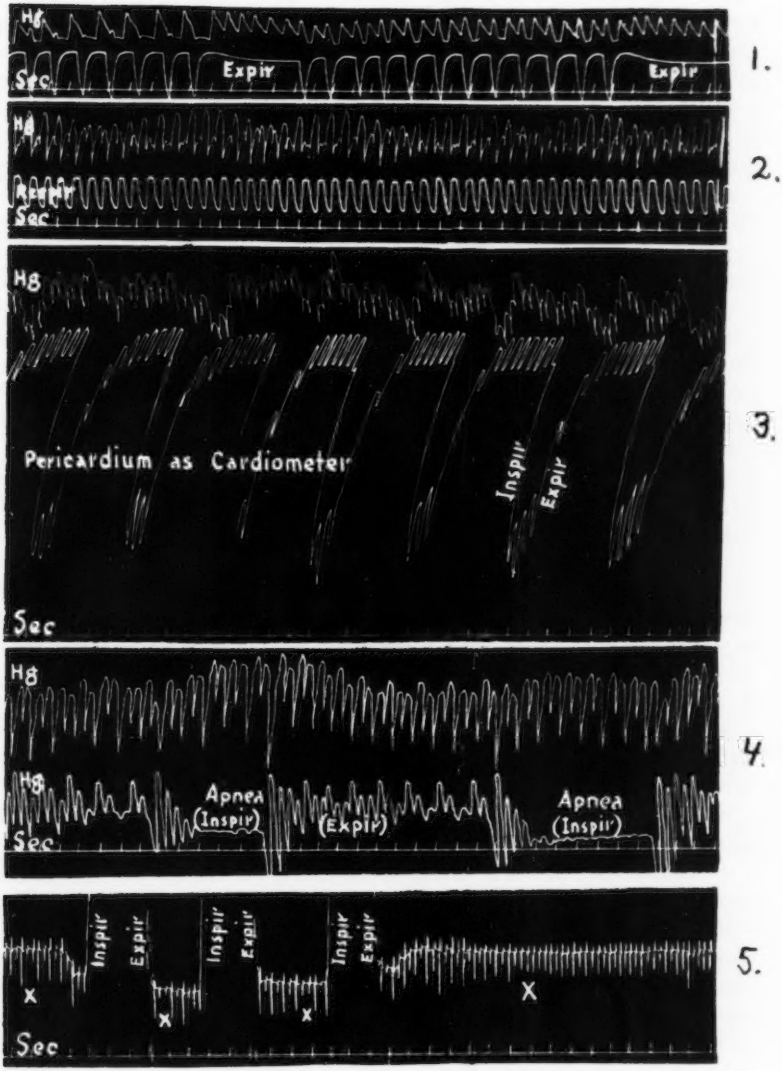
Fig. 1. Blood pressure, upper tracing, obtained with mercury manometer from left carotid of a dog. Shows two types of alternans, the nature of each type depending upon the timing of the artificial respirations. Inspirations almost completely eliminated three waves in first part of tracing. Middle tracing, artificial respiration by altering negative pressure in chest with suction pump. Recorded with Marey tambour attached to side arm of ether bottle. Down-stroke means inspiration. The dog was in apnea.

Fig. 2. To illustrate second training method. Respirations are natural. Respirations recorded with muscle lever connected to ensiform cartilage by means of a thread. Down-strokes are inspirations.

Fig. 3. Blood pressure tracing, upper, obtained with mercury manometer from felt carotid. Cardiogram, middle, obtained by cannulating pericardium and connecting to a sensitive tambour. It shows respirations as well as heart beats. The alternans is superimposed on the larger variations in both the blood pressure and volume curves. Respirations were spontaneous. Tracings were obtained after the dog was trained systematically for more than two hours by means of the suction pump.

Fig. 4. Upper tracing, blood pressure, obtained with mercury manometer from left carotid. Middle tracing, respirations, obtained with mercury manometer connected with chest cavity. *Inspir.* means lungs were greatly inflated by means of the suction pump. *Apnea* means absence of spontaneous movements of diaphragm and intercostal muscles; the small fluctuations in the curve during apnea are partly mechanical and partly heart action. *Expir.* means expiration relative to the artificial inspirations. The respiratory waves are complicated by heart beats and vibrations of the mercury column. There are 10 respirations indicated between the periods of apnea. The heart was trained by regulating the spontaneous respirations and by mechanically inflating the lungs.

Fig. 5. Heart beats and respirations obtained with a light lever attached to left carotid without occluding the artery. Each *X* means a transition in the alternans. The amplitude of the respiratory waves was about 7 cm.; the tops of these were cut off to save space. Tracing was obtained about four hours after the training began. Heart was trained by mechanically regulating the intrathoracic air pressure.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RESULTS. *Systematic training of the heart.* Figure 3 was obtained after the respiration-heart ratio was maintained, by means of the suction pump, at approximately 1:2 for slightly more than two hours. When the rapid artificial respiration ceased and the normal negative pressure was restored in the chest the dog breathed in a slow tempo, as is shown in the volume tracing. The alternans may be easily detected as an effect superimposed on the large respiratory variations in the arterial pressure tracing. It is also shown in the cardiogram. It is indicated by two signs in the blood pressure; the waves are alternately large and small and the larger of these are of greater duration than the smaller ones. In the volume tracing there is at times little and at times no difference in amplitude of the alternate waves, but there is a marked difference throughout the tracing in the duration of the alternate cycles. Direct observation of the heart showed not only that all of these waves mean ventricular systoles but that half of the heart beats were stronger and also of greater duration than the others. The failure of the cardiogram to show differences in amplitude of the waves at certain times is apparently a problem in mechanics rather than one in physiology.

In figure 4 a genuine alternans is present throughout the blood pressure tracing, but it is interfered with some between apneic periods by the respirations which did not occur at the appropriate rate at this time to augment the alternans effect. Above the first period of apnea every fourth beat is also especially emphasized. The first and second training methods were applied to this animal. The concentration of the ether vapor in the inhaled air was so regulated that a respiration-heart ratio of approximately 1:4 was maintained for almost three hours, and during this time a second respiration-heart ratio of 1:2 was maintained by artificially inflating the lungs. The groups of four beats during the first period of apnea in the tracing can probably be attributed to the 1:4 ratio. Such groups occurred many times in the records from this animal. The temporary apnea resulted because of sudden distention of the lungs by artificially increasing the negative pressure in the chest. It is interesting that the alternans occurred between as well as during the longer periods of apnea. It should be emphasized, in this work especially, that apnea is a relative term; it may mean only a slower breathing than at other times. Relative to the rate at which the dog was caused to breathe during the training there is relative apnea throughout this tracing; it is only greater at some places than at others.

Figure 5 shows one of several successful attempts at recording the heart action without using the mercury manometer and without partially or totally occluding any of the large arteries. A short, thin-walled glass tube was securely fixed in the left carotid artery, and a thread from this tube was passed in the forward direction over a small pulley and then

down to a light lever. The glass tube served the single purpose of preventing distortion of the artery when the thread was attached to it. The suction pump was used to maintain a respiration-heart ratio of approximately 1:2 for about two hours before any effects of the training could be detected. During the greater part of this time the ratio of the dog's own respirations to its heart beats was approximagely 1:4. Relative apnea, as shown in the tracing, could be produced by increasing the concentration of the ether vapor and not by strongly inflating the lungs with the suction pump. Transitions were frequent during the apneic periods. Some of these were sudden and others gradual processes. At certain times every fourth as well as every second beat was emphasized. This complex result is presumably the result of the two ratios, 1:2 and 1:4, during the training.

Effect of irregular respirations upon the heart. The spontaneous and artificial respirations of seven dogs were altered as frequently as possible. The negative pressure in the chest was changed at various rates, and at the same time the concentration of the ether vapor in the inhaled air was changed continually. The dogs stood this treatment for various periods ranging from almost one to about four hours. The results obtained during very slow breathing and special periods of apnea were generally simple but were too complex to interpret in a few instances. It is certain that there was no genuine alternans in any of the tracings.

Effect of very rough treatment of the heart. The hearts of three dogs were as badly mistreated as seemed possible without actually mutilating them. From time to time such a powerful suction was created in the funnel and chest that the lungs and heart were jerked up into the funnel where they remained for several seconds. The heart sometimes stopped beating on these occasions but beat feebly again when it fell back into the chest. In the course of thirty or forty minutes the hearts were very much dilated and appeared to be greatly weakened, but none of them showed a genuine alternans.

Blood pressure, heart tone and alternans. Both the blood pressure and the tonic condition of the ventricles varied greatly and independently in the same and different animals when both the apparent and the real alternans was best. The alternans was considered good when the alternate waves in the tracings differed considerably in amplitude. The absence of any definite relationship between any of the these phenomena was quite unexpected, but the observations were made with special care and were confirmed repeatedly.

Discussion. Our habit of associating the alternans type of beat with a weakened condition of the heart appears to be an outcome of observations which were made without regard to the previous influence of the respirations on the organ and perhaps also without the use of infallible

criteria for heart weakness. If a great loss of tone of the cardiac muscle means a weakened heart, it is peculiar that the mean blood pressure and the pulse pressure may or may not be exceptionally great when the ventricles are dilated sufficiently to completely fill the pericardial sac at diastole. If the alternans is itself a sufficient criterion for heart weakness, neither the blood pressure nor the tonic condition of the ventricular muscle can be considered as indicating a weakened heart because both of these were observed to vary greatly, together or independently, while either the apparent or the real alternans was good. It does not even appear useful to consider either a slow heart rate or a feeble beat as an absolutely dependable criterion because a slow rate may change suddenly to a more rapid one and a weak beat may change suddenly to a strong one. The evidences of heart weakness appear, therefore, to be largely speculative in nature, as might be expected when a very complex organ is the object of interest. In this work, especially, it seems best to talk as accurately as possible about the different types of reaction of the organ without speaking of heart weakness.

It was assumed that the heart can be trained, and it was almost proved that a number of hearts were trained to manifest the alternans type of reaction An observation which might appear to discredit the training theory is that excised mammalian hearts sometimes show not only alternans but transitions as well (Swindle, 1922). The simple alternans and transitions are characteristic reactions which indicate the capabilities of the heart. They are shown only rarely by the untrained but persistently by many of the trained hearts. It must be considered that the after-effects of the respirations may possibly be present in the excised heart as well as in the intact heart during apnea.

Training the heart appears to be like training a seal. The wild seal can and sometimes does balance objects on its nose. A little training may cause the animal to perform this act very frequently while all efforts to train it to be oviparous would be wasted. The heart can likewise be trained to do those things frequently which it is potentially able to do and which it may actually do at times without being trained.

There are two outstanding facts which support the training theory. One is that after the respirations are manipulated so that they affect the heart at two different rates at the same time, the heart may show two types of grouped beats at the same time. That is, every second but especially every fourth beat may be emphasized if the ratio of the artificial respirations to the heart beats was 1:2 while the ratio of the animal's own respirations to the heart beats was 1:4. The fact can not be easily dismissed that the quantitative nature of the resulting pattern of beats is predictable if the details of the specific training method are known. The other outstanding fact is that irregular artificial and spontaneous

respirations did not result in an alternans but only caused the heart to react feebly and then die. Some of these hearts were treated as roughly as possible without actually mutilating them.

When the respirations interfere with the heart beats interference waves in the blood pressure result. Two more or less independently acting mechanisms or functional units are required to produce such an interference phenomenon. In this case the interference is between the respirations and heart beats. It is scarcely conceivable that a transition of the kind here studied can be the result of the action of an organ which functions as a unified whole without being acted upon at regular intervals by some extrinsic force. The organ must therefore function in parts in order to show the transition during apnea. These parts must function alternately in two different respects; there must be the simple alternate action of the parts, and there must be for a time relatively weak action and then for a time relatively strong action of each functional unit to cause the transition. This is offered as an explanation for the fact that the heart may show both the alternans and the transition when there are no respirations or other detectable forces to modify the action of the organ at the time. Either of these units must be assumed to be powerful enough and also sufficiently well distributed in the walls of the ventricles to cause the entire ventricular mass to appear to contract while much of it actually rests. The alternate action of the units must be assumed to take place in the untrained heart even though the beats are then equal or about equal in strength.

The possibility should also be considered that there are many reserve units in the heart which act from time to time (paroxysmal tachycardia) when the ordinary units that normally keep up the circulation either cease functioning or become only less active. The respirations may be properly timed to cause a unit with a characteristically slow tempo of reaction to respond while the other units are reacting as usual. We might then observe that every fourth heart beat is peculiar in some respect, perhaps as in figures 4 and 5. It is conceivable that the heart is comparable in complexity to the central nervous system in which there are indications of division of labor, inhibition, augmentation and so forth.

CONCLUSIONS

1. Systematic regulation of artificial and natural respirations caused the heart to beat in certain predictable ways both before and during periods of apnea. The most important result was the genuine pulsus alternans in which characteristic transitions were present. The transition is the fundamental and the simple alternans is the incidental alternans type of reaction of the heart. An explanation of the transition must

necessarily be an explanation of the simple alternans as well, but the reverse may not be true.

2. The method of systematically regulating the respirations is a fundamental training method for the heart.

3. Irregular respirations did not cause the heart to beat in the alternans fashion.

4. Rough manipulation of the heart caused it to beat feebly and die without showing any alternans.

5. As a rule direct observation of the heart showed that the results in the tracings were genuine and not due to instrumental error. Only the volume curves of the ventricles were sometimes inaccurate.

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THE CHEMICAL SENSITIVENESS OF THE KIDNEYS[1]

EDWARD F. ADOLPH[2]

From the Zoölogical Laboratory, The Johns Hopkins University

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To describe what the kidneys do, it is necessary to find the relation between stimulus and response. This is the usual procedure in investigating most living units, and in evaluating any artificial contrivance; the first step is always to correlate the output with the factors controlling it.

Many stimuli or factors once supposed to be significant in the formation of urine by the mammalian kidneys have been shown in the work of the past 85 years to be of no importance. It is now legitimate to affirm, as Starling does (Dreyer and Verney, 1923), that only two factors are of importance in determining the performance at a given time of a given pair of kidneys: the blood pressure and the chemical composition of the blood. Of course each of these two factors is made up of innumerable combinations of constituent variables. But from the work of Addis and Drury (1923a) we now know that it is possible to keep all the variables either constant or counterbalanced, so that under defined conditions one can investigate the influence of a single change in the chemical composition of the blood upon the rate of excretion of certain substances. Because these substances are specifically excreted as chemical individuals by the kidneys, their excretion constitutes a measurable response on the part of the kidneys. And because the concentration of each substance in the blood determines the response, we can evaluate the chemical sensitiveness of the kidneys.

For many years it was supposed that by comparing the concentration of each constituent of the blood plasma with the concentration of each same constituent in the urine, one obtained a measure of the kidney's performance. But it was eventually found that usually the urinary concentrations were independent of the corresponding blood concentrations, and indeed that when some one substance is copiously excreted, its concentration in the urine becomes maximally constant for all rates of excretion (Ambard and Papin, 1909). Nevertheless, the study of relative concentrations served to emphasize the fact that all the chief constituents of

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[2] National Research Council Fellow in Zoölogy.

the urine are present in higher concentration in the urine than in the corresponding blood. Exceptions to this are the interesting cases of those substances which pass into the urine by ordinary diffusion and are therefore present in identical concentrations in blood and in urine. Such substances are methyl, ethyl, and propyl alcohols, ethyl acetate, acetone and chloroform (Widmark, 1916; Chabanier and Ibarra-Loring, 1916; Chabanier, 1917; Widmark, 1920; Briggs and Shaffer, 1921).

Only in recent years, however, has it been realized that the rate of excretion, and not the urinary concentration, is the proper measure of the kidney's activity toward a particular substance. This proposition was first given a high degree of prominence by the attempt of Ambard (1910) to relate quantitatively the rate of excretion of urea with the blood concentration of urea. The true relation for urea has been shown by Addis, as the result of several years' study, to be for urea a very simple one, and a readily reproducible one under a given set of conditions.

From the study of certain extant data we believe that a reproducible relation exists for urea under a second set of conditions which differ from those of Addis, and this relation itself differs in a slight but regular way from that of Addis. From an analysis of further published data, we find definite relations also in the case of phosphate excretion; and the relations for phosphate and perhaps also for other substances appear to be identical in certain respects with those for urea. From these findings we arrive at the suggestion that the excretion of various substances is accomplished by one kind of process.

THE RATE OF UREA EXCRETION. In a set of beautiful experiments upon man, Addis and Drury (1923a) showed that the rate of urea excretion, D, is directly proportional to the concentration of urea in the blood, B. Using r as a proportionality constant, we may then write $D = rB$. The conditions under which this relation holds they describe as follows: Upon arising in the morning the subject drinks one liter of water containing an amount of urea. Every hour thereafter he drinks a half-liter of water. No food is taken, and bodily activities are slight. The first measurements are made at least three hours after the ingestion of urea; the urine is collected hourly, and samples of blood are drawn from a vein at the middle of each urine period.

Upon analysing the available data relating blood concentrations to rates of urinary excretion, we find that there is another set of conditions where concordant results have been obtained. The most satisfactory data for urea excretion are shown in figures 1 and 2; in one case the data were obtained by Hewlett, Gilbert and Wickett (1916) upon the human subject Hew., and in the other case the data were obtained by McEllroy and Pollock (1921) upon two dogs. In both cases the experiments were performed for other purposes, and their observers therefore did not analyse

very far the correlation between the blood concentrations and the rates of excretion. In figures 1 and 2 it can be seen, however, that the several points can be correlated in only one way; that is, by drawing a straight line through them.

But the straight line in neither case passes through the origin, so that the formula for this relation is: $D = r(B-a)$. Since the line having the slope r cuts the abscissa, the data clearly suggest that the urea excretion may be zero at a finite blood concentration a. In other words, a represents a threshold for urea excretion.

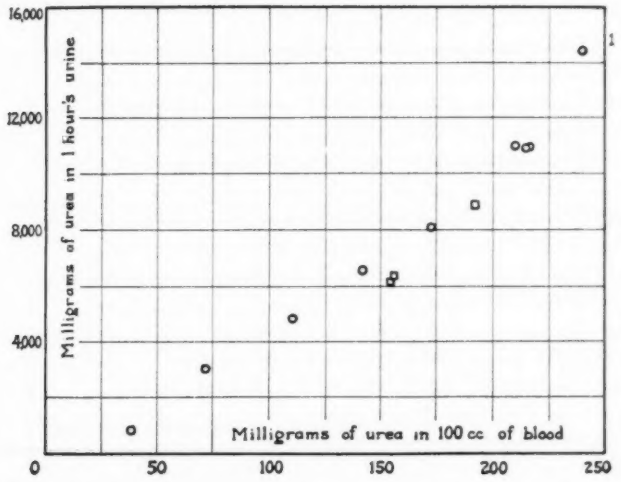

Fig. 1. Correlation of concentrations of urea in whole blood with rates of excretion of urea in urine. Experiment upon the human subject Hew. after the ingestion of 100 grams of urea. The circles are points obtained while the blood concentration was increasing, the squares are points obtained while it was decreasing. All the points except the highest are correlated by a straight line which cuts the abscissa at the value of 22 mgm. per 100 cc. Data taken from Hewlett, Gilbert and Wickett (1916).

The conditions under which this relation holds we may describe as follows: A single large ingestion of urea (Hewlett) or of meat (McEllroy) is made without an excessive water intake. No special precautions are observed except that muscular activity is limited. Samples of urine and blood are collected hourly, and their collection may start with the ingestion. The data therefore represent the kidneys' activities on a *single* day, during the excretion of an *excessive* amount of the one substance, urea.

That these results can be reproduced with fair agreement is indicated by the fact that the data of a second experiment performed by Hewlett and his associates (1916) upon the subject Hew. are best represented by a

straight line which has a slightly greater slope than that for the experiment shown in figure 1; the deviation of the points from the line was somewhat greater than in the first experiment. The same authors also give single experiments upon three other men; in each case the data are correlated fairly well by straight lines all of which are quite similar in origin and in slope. The data for dogs are satisfactory so far as they are available, the only two protocols published by McEllroy and Pollock (1921) are represented in figure 2, and it is clear that the data of both are best correlated by the same straight line. We have no illusions as to the ease with which reproducible results are obtained, a point which Addis has con-

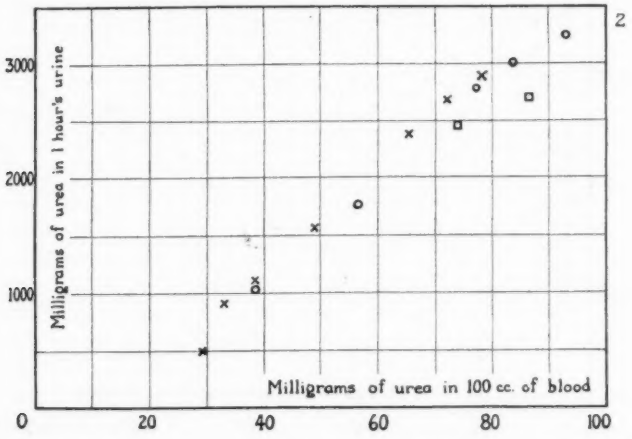

Fig. 2. Correlation of concentrations of urea in whole blood with rates of excretion of urea in urine. Experiments upon two dogs which were fed meat. Crosses, a single day's experiment upon dog XIV; all points were obtained while the blood concentration increased. Circles and squares, a single day's experiment upon dog XIII; the circles representing points obtained while the blood concentration was increasing and the squares representing points obtained while it was decreasing. Data taken from McEllroy and Pollock (1921).

stantly emphasized; we have found many sets of data useless for our purpose, and the absence of consistency in results, such as in those discussed by White (1923), is to be expected in conditions other than those specified. But we believe that where the conditions have been fulfilled by careful experiments, the data resulting may be taken to represent an accurate physiological evaluation.

We now have two conditions under which reproducible correlations may be obtained between the concentrations of urea in the blood and the rates of urea excretion. In one case the relation is expressed by the formula $D = rB$; in the other case by the formula $D = r(B-a)$. These formulae

and the actual values of r and a in the various experiments, we can compare by means of figure 3. There it is evident that the slope r of the lines of proportionality does not differ significantly under our conditions from that under the conditions selected by Addis and Drury (1923a). Only the origin of the lines differs, that is, the value of a. For Addis and Drury a is zero; for the other cases a is 22 mgm. in 100 cc. (Hew.), and 15 mgm. in 100 cc. (dogs XIII and XIV), respectively. In one set of conditions there is no threshold, in the other set there is a threshold.

The existence of the apparent threshold is correlated with the conditions, for we know from other data on Add. (Addis and Drury, 1923a) that he also "ordinarily" exhibits an apparent threshold. The chief difference in the conditions selected by Addis and Drury and those designated by us is in the amount of water ingested. As far as we can perceive, this is the only essential difference between the two sets of conditions. We suggest, therefore, that the threshold represents a resistance to be overcome by the kidneys in obtaining water for urea excretion. With a water diuresis averaging 500 cc. per hour this resistance no longer existed. A strikingly similar effect of water ingestion upon chloride excretion we found in a peculiar instance described elsewhere (Adolph, 1921, his figure 5). In this instance water during its absorption from the intestine was more available to the kidneys than at other times; such was evidently the case in the experiments of Addis and Drury.

Addis and Drury (1923b, 1923c) calculated the value of r under various conditions, upon the assumption that $D = rB$. If no urea was ingested, the values calculated for r were considerably lower, whether the urine volumes were ordinary or whether the urine volumes were large. Upon the basis of the evidence given above, we believe that their formula does not hold for these conditions; it is likely that the value of a was the chief variable in these diverse conditions.

That acute lack of water has a tremendous effect in decreasing the excretion of urea by rabbits was shown in Addis' laboratory by Mackay and Mackay (1924). We interpret this fact to mean that the apparent threshold increases markedly in thirst.

It seems worthy of remark that the data for urea excretion given by Ambard and Weill (1912), the very data for man and dog upon which they based their "law" of urea excretion, fit the relation $D = r(B-a)$ at least as well as their relation $D = rB^2$. The only justification for their formula, even at the time of its promulgation, was the supposition that there is no threshold for urea excretion. These data, and those of McLean (1915) and of Austin, Stillman and Van Slyke (1921), were not, however, obtained under the conditions which are required in order that accurate values of r and a may be calculated.

It is of interest to compare the rates of urea excretion, under the con

ditions chosen by us, for different species of mammals. In addition to the data for man and dog discussed above, we can legitimately compare with them certain data for rabbits given by Drury (1923). The rabbits received urea intravenously without additional water; we have selected the data for a rabbit (his rabbit 1) nearest the average, neglecting the points obtained without urea administration. In figure 3 are shown the excretory ratios for these three species. It is evident that both a and r differ among the species; a in mgm. per 100 cc. is 22, 15 and 40 respectively, while r, which is defined as $\frac{\text{mgm. urea in 1 hour's urine}}{\text{mgm. urea in 100 cc. blood}}$, is 54, 45 and 4.9 respectively.

The value of the "threshold" a is not correlated with any obvious feature of the kidneys; as far as we now know, it is arbitrarily fixed. But it is highly interesting that the normal values for urea concentration in the bloods of each of these species is correlated with the size of the threshold as determined by our method. The normal ranges of blood urea, in mgm. per 100 cc., are now well established as follows: man 25–35 (Myers, 1924); dog 15–55 (Haden and Orr, 1923); rabbit 35–80 (Addis, Barnett and Shevky, 1918b). In our opinion the existence of a threshold, when the organisms are not flooded with excessive water, determines the magnitude of the normal level of urea in the blood.

The value of the ratio r must be correlated to some extent with kidney size, and therefore with body size, as Taylor, Drury and Addis (1923) found true for rabbits. But it is obvious in figure 3 that the dog's ratio is enormous for its body size, compared with man's. If we calculate the ratios of the body weights to the values of r for these three species, the following individuals studied show the following comparative numbers: man 0.7, dog 3.7, rabbit 2.2. A closer correlation would be reached by comparing the values of r with the body surface instead of the body weight, as already suggested by Taylor, Drury and Addis (1923). Again, from general considerations it might be argued that r is a measure of the intensity at which a renal epithelium can work; the existence of a factor of this sort could be ascertained only if the areas of the effective epithelium could be equated and the blood pressures made identical.

In our opinion the most remarkable feature of the excretion of urea is that the proportionality between blood concentration and excretory rate can be maintained by the kidney at extremely high levels; in reality no limit has been found to the proportionality. Addis and Drury (1923a), with high water intakes, studied values up to 100 mgm. per 100 cc. in man. Hewlett, Gilbert, and Wickett (1916) attained 240 mgm. per 100 cc. in man (fig. 1). The dogs of McEllroy and Pollock (1921) reached 100 mgm. per 100 cc. (fig. 2). The rabbits of Drury (1923) showed the expected rates of excretion at 780 mgm. per 100 cc., which is very nearly the lethal dose. In fact, then, the kidneys never reach the limit of the velocity with which they may excrete urea; if the proper chemical stimulus

were presented, the predicted rate could presumably be attained at blood concentrations above the lethal level.

Apparently there is no fatigue on the part of the kidneys, for the ratios have the same value at the end of a day of high rates of excretion as they have at the beginning (figs. 1 and 2). The instances cited by Addis, Meyers and Bayer (1925) of changes with time in rate of urea excretion are in reality changes with blood concentration; they no longer constitute exceptions when one admits the existence of an apprent threshold under conditions of limited water ingestion.

In summary, it appears that under a second set of conditions, other than those used by Addis and Drury (1923a), accurate quantitative correlations may be made between the concentration of urea in the blood and the rate of its excretion. The existence of a *threshold* for urea excretion under ordinary conditions accounts for the values of the normal blood concentrations. The existence of a threshold depends upon the rate of water excretion. The excretory *ratio* is probably unchanged by the presence or absence of excessive water supply. The values of both the threshold and the ratio characterize the kidneys of each individual and each species.

THE EXCRETION OF OTHER SUBSTANCES. Although urea is the substance whose excretion has been studied most thoroughly, we have available fragmentary data concerning a few other substances.

Phosphate excretion has been studied in man by Wigglesworth and Woodrow (1924). They compared, after neutral phosphate ingestions, the blood concentrations with the rate of excretion, both with the body normally hydrated and with an excess of water present (2.5 liters taken during the day). Their data under the latter conditions are the most consistent, and are plotted in figure 4. The data for each of their two subjects are correlated well by straight lines, which do not pass through the origin. For both subjects the lines cut the abscissa, and in a single point. We thus have graphs which are represented by the equation $D = r(B-a)$, just as for urea excretion at ordinary hydration levels. For the two subjects Wig. and Woo. the value of a is 2.9 mgm. per 100 cc. of plasma; the value of r (in the same units as for urea excretion) is 54 for Wig. and 47 for Woo.

The ratio r has approximately the same values for phosphate excretion and for urea excretion in man.

Phosphate excretion has been studied in rabbits by Addis, Meyers and Bayer (1925). They injected neutral phosphate solutions into rabbits without excessive water supplies. All their data obtained under these conditions are represented in their figure 3, and in every case the best correlations are given by straight lines which cut the abscissa. For a single rabbit nearest the average (their rabbit 1), this line corresponds

to the formula $D = r\,(B-a)$, where a is 7.5 mgm. per 100 cc. of plasma, and r is 5.5 for an animal weighing 2.3 kilograms.

The ratio r has approximately the same values for phosphate excretion and for urea excretion in rabbits of the same body weight.

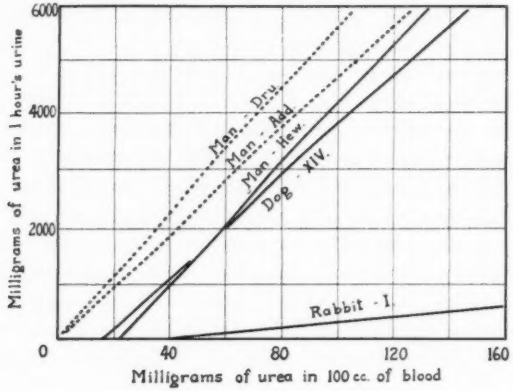

Fig. 3. Graphical comparison of excretory thresholds and ratios for urea excretion. In all cases urea or meat was administered in excessive quantities; in the experiments on Dru. and Add. water was also administered copiously. For numerical comparison and sources of data, see table 1.

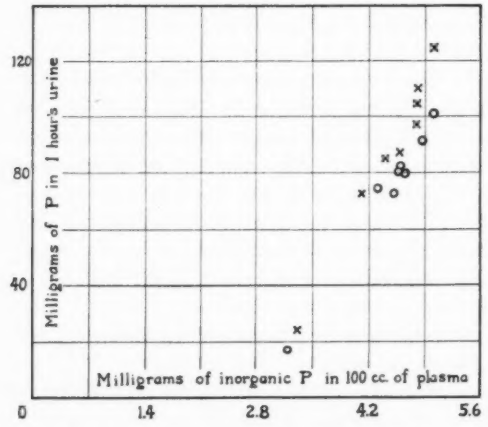

Fig. 4. Correlation of concentrations of inorganic phosphate in blood plasma with rates of excretion of phosphate in urine. Two experiments upon two human subjects after the ingestion of dibasic phosphate equivalent to 2.1 grains of P, and during the ingestion of 2.5 liters of water. Crosses, subject Wig.; circles, subject Woo. Data taken from Wigglesworth and Woodrow (1924).

Addis, Meyers and Bayer (1925) have avoided the conclusion that phosphate has a threshold of excretion. It is still possible, as they hypothecate, that under certain conditions the threshold might disappear. But under the conditions with which they worked, and under the conditions used by Wigglesworth and Woodrow (1924), there was always a threshold. Instances are now known in which the phosphate concentration in urine was lower than the concentration in blood. Moreover, the threshold was no lower in the subjects Wig. and Woo. when 2.5 liters of water were taken with the phosphate than when no extra water was taken. It seems probable that for phosphate, as for chloride, the threshold exists under all observable conditions.

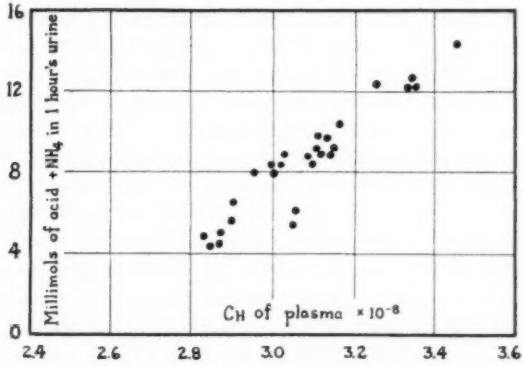

Fig. 5. Correlation of concentrations of hydrogen-ions in blood plasma with rates of excretion of titratable acid plus ammonium. Several experiments upon the human subject Hal. after the ingestion of ammonium chloride. The C_H of the plasma was actually measured as alveolar tension of CO_2. The points are correlated by a straight line which cuts the abscissa at the C_H value of 2.5×10^{-8}N. Data taken from J. B. S. Haldane (1921).

It is interesting that both the threshold for urea and the threshold for phosphate are considerably higher in rabbits than in men. The values of the thresholds for the two substances bear no obvious relation to each other, however; they are not equimolecular. Certain data of White (1923) indicate that the phosphate threshold in dogs is lower than in rabbits, which is parallel to what we found above for the urea threshold in these two species.

Acid excretion was studied in man by J. B. S. Haldane (1921) after the ingestion of ammonium chloride. He compared (in his figure 1) the rates of excretion of titrable acid plus ammonia with the prevailing alveolar tensions of carbon dioxide. We have reproduced his data, plotted to other units, in figure 5. It will be seen that these data are correlated well

by means of a straight line. By extrapolation of this line we find that the threshold for acid excretion is an alveolar CO_2 tension of 46 mm.; this corresponds to an arterial hydrogen-ion concentration of 2.5×10^{-8} N (pH = 7.41), assuming that the alkali reserve of the subject Hal. did not change during his experiments. As far as we are aware, the threshold of acid excretion has not been previously estimated, and we are not convinced that it has any general validity.

For acid excretion we can also use the formula $D = r(B - a)$. But the evaluation of r is meaningless here except as an empirical number, for we know regarding the blood only the C_H, and regarding the urine only the titrable acid. We have no means of ascertaining, therefore, whether this r is in reality equal to the r for the excretion of urea and of phosphate by man.

Creatinine excretion has been studied experimentally in dogs by Fonteyne and Ingelbrecht (1923). They give data for only one dog, which was injected with 1 gram of creatinine; but it happens that these data are correlated well by means of a straight line, $D = r(B - a)$, where a has the value 1.0 mgm. per 100 cc. of whole blood, and r the value 40. We mention these data with great reserve, because Behre and Benedict (1923) doubt whether creatinine can be determined, at least at normal levels, in mammalian blood even by experienced hands. We can avoid this dilemma by stating that if it is not creatinine, it is the substance measured as creatinine for which the data hold. It is certainly worthy of note that the value of r for this substance in the dog is very nearly equal to that for urea in the dog, as given above. The value of a is what one should expect from the normal range of 1.1 to 2.1 mgm. per 100 cc. found in dogs by Haden and Orr (1923).

Chloride, though one of the chief substances excreted, has not been studied heretofore by the method of finding the excretory ratio after copious ingestion of it. The data of McLean (1915) upon man were not obtained under conditions at all comparable to those where we find a correlation possible. The data of Austin and Jonas (1918) and of Whelan (1925) upon dogs are also not comparable. Without copious chloride administration and without frequent periods of urine collection and blood sampling, it is quite impossible to obtain data utilizable for our purpose. Such data, however, Doctor Addis informs us, have recently been obtained in his laboratory upon rabbits; we eagerly await their publication to see whether or not the value of r for chloride is comparable to the values of r for urea and for phosphate.

It may be noted in passing that the calculation of threshold values, particularly in the case of chloride, has been made to depend (Ambard, 1920; McLean, 1915) upon the excretory ratio. Since these authors used formulae for the excretory ratio which we now know are erroneous,

it follows that the values of the thresholds must be recalculated. For chloride we have at present no data from which to calculate the threshold accurately. It should be emphasized in this connection that under many conditions, particularly under ordinary conditions, the blood level may be very slightly below the true threshold value. We now know, also, that a threshold has an assignable magnitude only under a range of conditions which must be specified.

For the excretion of uric acid, sulfate, potassium, calcium and magnesium, no data are available. We expect to find in each case, however, a small but real threshold under ordinary conditions of hydration, when the graphs correlating blood concentration and urinary excretion at rates above ordinary are extrapolated. Data for dextrose and other sugars have apparently not been worked out under the conditions which we specify, and for no sugar do we know the value of r. For foreign substances such as phenolsulfonephthalein, iodide and boric acid we are entirely without information regarding excretory ratios. For bicarbonate the data are merely sufficient (Davies, Haldane and Kennaway, 1920) to indicate that the rate of excretion is a function of the C_H of the blood.

Water is the substance about whose excretion we know least of all. Attempts to correlate diuresis with blood dilution have, in nearly all cases, yielded only qualitative results. Priestley (1921) has obtained even qualitative results with only three out of nine methods which he used for estimating changes in the water concentration of blood after copious water injection. The failure of physical methods to rival the kidney's measurement of blood dilution has led many, as Starling and Verney (1925), to the view that there is no excretory ratio for water. But knowing that for other substances there are many factors influencing the excretion, it seems premature to conclude that water is not specifically excreted in water diuresis as a quantitative response to changes in the water content of the blood.

Discussion. As already pointed out, the cases are very few in which the data upon rates of excretion by the kidneys are adequate for the calculation of the excretory ratios. Data upon a very few individual organisms were found to be sufficiently complete for the analysis of the excretion of urea and of phosphate. These data, together with the less adequate data upon acid and upon creatinine are summarized in table 1. It may be recalled that the data for urea are extensively confirmed as to their general magnitude by the earlier data of Addis and Watanabe (1917) upon man, of Marshall and Davis (1914) and Austin, Stillman and Van Slyke (1921) upon dogs, and of Addis, Barnett and Shevky (1918a) upon rabbits.

The conclusions to be drawn from the sifted evidence, as it is represented in table 1, are as follows: (*a*) under two separate sets of conditions exact data upon the rate of excretion are obtainable. The conditions in these

TABLE I

Values of the excretory ratios r and of the thresholds a in cases where reliable data have been obtained upon the excretion of various substances

The relationship between rate of excretion and blood concentration is expressed by the general formula $U = r\,(B - a)$.

SUBSTANCE	SUBJECT	BODY WEIGHT	AUTHOR	r	a	RANGE OF CONCENTRATIONS FOUND IN NORMAL BLOODS
		kgm.			*mgm. per 100 cc.*	*mgm. per 100 cc.*
Urea	Man, Add.		Addis and Drury (1923a)	46.0	0	
Urea	Man, Dru.		Addis and Drury (1923a)	56.0	0	
Urea	Man, Hew.	75.0	Hewlett, Gilbert and Wickett (1916)	54.0	22	25–35
Urea	Dog, XIII and XIV	12.0	McEllroy and Pollock (1921)	45.0	15	15–55
Urea	Rabbit, 1	2.3	Drury (1923)	4.9	40	35–80
Phosphate	Man, Wig.	70.0	Wigglesworth and Woodrow (1924)	54.0	2.9	2.5–3.3
Phosphate	Man, Woo.		Wigglesworth and Woodrow (1924)	47.0	2.9	
Phosphate	Rabbit, 1	2.3	Addis, Meyers and Bayer (1925)	5.5	7.5	
Acid + NH_3	Man, Hal.	97.0	Haldane (1921)	—	2.5×10^{-3}N	2.8×10^{-3}N
Creatinine	Dog, 1		Fonteyne and Ingelbrecht (1923)	40.0	1.0	1.1–2.1

two sets differ primarily in respect to the amount of water simultaneously excreted. The results obtained under these two sets of conditions differ only in respect to the existence or non-existence of a *threshold*. (*b*) For a given mammalian species the excretory ratios are equivalent for urea and for phosphate (and possibly for creatinine). For acid and chloride the evidence is not to the contrary. (*c*) The apparent thresholds of excretion differ for each substance and for each species. But for both phosphate and urea they are respectively higher in the rabbit than in man.

The meaning of the apparent threshold for urea requires discussion. From the studies which have been made by Addis we know that this threshold is abolished by simultaneous addition of copious water to the body. We believe that the threshold is related to the quantitative difficulty of withdrawing water from the body for excretion. However this may be, the threshold is at least a *threshold of stimulation*. At or below the threshold value there is some elimination, in fact "normal" human urine nearly always contain more urea than is to be expected from the formula $D = 54\ (B - 22)$ which is that found to hold for the subject Hew. Such normal urines constituted the material for the excretory ratios in man analysed by McLean (1915) and by Austin, Stillman and Van Slyke (1921).

We believe that the higher excretions of urea exhibited after copious intake of both water and urea are in part due to a superadded "washing out" or augmenting process in which the kidneys are passive or unstimulated. The amount of this augmentation is not necessarily related to the volume of the urine, except at low rates of water excretion, that is, at rates such as normally occur. The demonstration of Addis and Drury (1923c, their figure 3) that the urea excretion is largely independent of the urine volume in reality confirms our view, for their lowest volumes showed a considerable retention of urea while a maximum of elimination was maintained immediately thereafter.

In an earlier paper (Adolph, 1923a) we have demonstrated that in most normal urines the amount of water excreted is determined or "obligated" (Ambard and Papin, 1909) by the chloride to be excreted. In such urines urea passes through the kidneys in excess of that which the kidneys actively excrete. Thus normal urines contain amounts of urea which for the subject Hew., for instance, would lie between $D = 54\ (B - 22)$ and $D = 54\ B$. It must be evident that the existence of such a threshold under conditions where water is not present in excess of the body's level of retention, constitutes a considerable economy to the kidneys. With such a threshold of stimulation the kidneys are thrown into secretory activity only for a certain proportion of all the molecules of various kinds which are eliminated; most of them are carried through in the obligatory volume.

We believe that his conception of a threshold, which probably applies

to the other substances besides urea which are ordinarily considered to be non-threshold substances, explains many of the departures from consistent excretory ratios which were found by Addis and Watanabe (1917) and others under standardized conditions, and particularly under ordinary conditions where no urea was ingested. The only criteria which remain by which we can distinguish between a true threshold such as that usually existing for chloride and an apparent threshold such as that for urea, are: (*a*) showing by appropriate experiment that copious water ingestion does not remove the threshold, and (*b*) finding urines in which the concentration in the urine is less than that of the blood. We believe that for phosphate Wigglesworth and Woodrow (1924) have by both these criteria shown the threshold to be a true one.

The conception of an apparent threshold also explains the magnitude of the concentrations of urea and such substances normally found in blood. It has long been known, for instance, that the urea concentration of human blood rarely falls below 20 mgm. per 100 cc. The usual explanation has been that the kidneys could not remove the substance faster than this, since its production was continually going on. But it must be clear now that the kidneys usually become inactive at this concentration in man. Below such concentration urea is removed from the blood very slowly, as Bollman, Mann, and Magath (1924) have shown in a dog with extirpated liver. Their data show that the ratio for urea excretion no longer holds at these low concentrations; that the elimination is passive and never complete. To us it seems quite inconceivable that the composition of human blood plasma should be constant in all individuals at so high a concentration, unless elimination slows down at some threshold. That normal urea values in whole blood should nearly always fall between 25 and 35 mgm. per 100 cc. (Myers, 1924), or that normal phosphate values in plasma should fall between 2.5 and 3.3 mgm. per 100 cc. (Tolstoi, 1923), indicates that there are ordinarily narrow ranges of variation and these must mark the boundaries between inactivity and activity on the part of the kidneys.

The chemical sensitiveness of the normal kidneys must represent a large number of accurate adjustments. On one hand, too great a sensitiveness would require needless expenditure of work for specific excretion, and at times would lead to the elimination of valuable materials, such, for instance, as would occur if the alkali reserve of the blood were reduced during a brief forced breathing. Too small a sensitiveness, on the other hand, would confer a grave degree of variability to the body's internal medium which would not be rapidly corrected. In both directions there are large margins of safety.

It is known that the sensitiveness of the kidneys is varied by a great many factors in addition to the concentration of the substance to be

excreted. The most accurate measurements of some of these factors have been made upon the rate of urea excretion by Addis and Drury (1923b). Haldane and his associates (1921, 1924) have shown that phosphate augments the rate of acid excretion and that acid augments the rate of phosphate excretion. In none of these or of numerous other studies including those of diuretic drugs is it clear how much the excretory ratios are influenced and how much the thresholds are varied. The only way to find out the nature of these factors is to work out complete curves of at least six points, adding the influencing substance to the body in equal hourly doses.

It is clear that all the thresholds and ratios are intimately bound up together in the kidneys. Thus Gross (1923) has shown a tremendous reduction in the thresholds for Na and Cl after the administration of oxalates to dogs. The chemical sensitiveness of the kidneys, (that is, presumably, the values of a and r) has yet to be defined, therefore, for a case where more than one essential variable is introduced. It is clear that what the kidneys are doing is to adjust the concentration of each chemical individual in the blood *relative to* the concentrations of many or all of the other chemical individuals present.

The fact that r was found to have equal values for urea and phosphate in men, for urea and phosphate in rabbits, and possibly for urea, creatinine and chloride in dogs, suggests more forcibly than anything yet brought to light, that there may be a mechanism common to the excretion of several substances. Though the kidneys never fail to distinguish between them, yet the quantitative relation between stimulus and response is identical. In other words, it is possible that the type of work performed is the same in the several cases.

To speculate upon the physical nature of the kidneys' work is highly premature; but we cannot refrain from the remark that in the kidneys the response or performance obviously is related directly to the stimulus. Let us suppose that as the blood flows through the capillaries in the kidneys urea escapes from the blood in proportion to its concentration. Then the quantity of the material received for excretion would be identically the stimulus. If it is of mental assistance, one may say that the transfer of urea from the blood into the renal epithelium corresponds to Fick's (1855) "law" of diffusion. This, then, implies the assumption that whatever gets into the renal epithelium is formed into urine as rapidly as it is received.

We were interested to see whether other chemically sensitive tissues showed a relationship between blood concentration and response similar to that shown by the kidneys. The sensitiveness of the respiratory center has been thoroughly investigated by Campbell and his associates (1913, 1914), Peabody (1915) and Scott (1917). The rate at which CO_2 is expired

from the lungs was in all cases a function of the percentage of CO_2 inspired, but not a simple linear function. The relationship requires a formula sufficiently complex that it cannot receive a physical interpretation, as de Almeida (1923) has shown. We were, however, interested in the fact, which we have frequently observed, that a small percentage of CO_2 in the inspired air produces almost no increase in the breathing. A considerable increment is required to affect the respiratory center measurably, roughly simulating a threshold condition.

The only other tissues whose sensitiveness to changes in chemical concentrations of the blood has been measured quantitatively are glands. Denis and Sisson (1921) obtained data which indicate that the volume of milk produced by goats is reduced in an anhydremia due to the administration of sodium chloride. Starr (1922) showed that the acidity of the saliva varies in relation to the carbon dioxide tension of the blood. Morris and Jersey (1923) have correlated changes in the water and other substances secreted by the salivary glands with experimental alteration of blood composition. We have found (Adolph, 1923b) changes in the acidity of sweat during the progressive alkalosis caused by exposure to heat. The ultimate quantitative relationships in these glands are, therefore, still unknown; it is, of course, obvious that the work of glands other than the kidneys is to minimize rather than to exaggerate in their products the chemical influences of the blood.

SUMMARY

1. From data published by other investigators it is demonstrated that accurate correlations may be made between the blood concentrations and the rates of excretion of some one ingested substance. When no extra water excretion occurs, a slightly different type of reproducible result is obtained than Addis and Drury (1923a) obtained with high water intakes. The chief difference is found to lie in the existence of an apparent threshold for the excretion of other substances.

2. The apparent thresholds and the excretory ratios are compared for three different mammalian species and for four different excreted substances (table 1). In all cases, under the conditions of experiment specified by us, there is a threshold or range of blood concentrations within which the kidneys are insensitive.

3. Under ordinary conditions substances are often carried through the kidneys by a passive process. Thus materials are eliminated in addition to the amounts actively secreted in response to the stimulus of the blood concentration of each chemical material.

4. The recognition of a threshold having a constant value for specified conditions makes possible the accurate estimation of the excretory ratio. The ratio is an expression of the fact that under the given conditions the

rate of excretion is directly proportional to the blood concentrations of the substance in question.

5. It turns out that the excretory ratio has the same value for urea and for phosphate, and possibly for other chemical individuals. It is therefore conceivable that the methods of their excretion are similar.

We are indebted to Dr. T. Addis of Stanford University for the opportunity of discussing with him certain of the questions involved in this paper.

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NOTE ON THE RELATIONSHIP BETWEEN VOLUME AND REACTION OF URINE SPECIMENS

ROGER S. HUBBARD

From the Clifton Springs Sanitarium and Clinic, Clifton Springs, New York

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In a series of articles recently published from this clinic (Hubbard and Munford, 1922; Hubbard and Allen, 1923; Hubbard, 1924) the reactions of urine specimens collected at hourly intervals have been compared with the amounts of titratable acid and ammonia which they contained and the volume of fluid excreted. In each series of results reported there was a tendency for the reaction of specimens with large volumes to be more alkaline than was that of those with smaller volumes. The opinion was advanced that this finding did not depend on an essential relationship between the volume and reaction of urine specimens, but that an increased excretion of fluid coincided with the alkaline tide after meals. It has seemed worth while to study the results further to decide whether the volumes and reactions of urine specimens tend to vary together.

The methods used have been previously described (Hubbard, Munford and Allen, 1924). The material presented includes all specimens collected for an hour which have been previously discussed, and of a number of specimens from unselected cases which had been previously studied by the fractional gastric analysis method and upon which determinations of the presence or absence of an alkaline tide were made. None of the subjects was receiving alkali or acid when the studies were carried out. Most of the analyses were done on urine specimens collected between 7 a.m. and 1 p.m. The data are presented in figures 1, 2 and 3.

In figure 1 are given the volumes and reactions of all specimens studied except two series upon normal subjects which are given later. The average acidity decreased somewhat as the volume increased, but the distribution of pH values of specimens with volumes from 10 cc. to 250 cc. shows that there was no essential relationship between the volumes and reactions of specimens. There were specimens with large volumes and a marked degree of acidity, and also alkaline specimens which had small volumes. It is possible that when the volumes of specimens were very small there was some tendency for their acidity to be high, but even with hourly volumes of less than 20 cc. there were a number which showed low acidity. No great accuracy can be claimed for the results when the reactions were

alkaline as the specimens were collected in the usual way, and it has been shown that changes in the carbon dioxide in such specimens affect the reaction (Marshall, 1922). The differences shown between the values found on different specimens are in many cases too great to be explained on the basis of such changes. It is probable also that when the volumes were very large the results were not strictly comparable with the others because of differences in the amounts of salts present.

In figure 2 are given the results of repeated studies upon two normal men which have been previously reported in a different form (Hubbard and Munford, 1922; Hubbard and Allen, 1923). Here too there is shown some diminution in average acidity when larger volumes were excreted, but the distribution of the values of pH and volume shows that there was

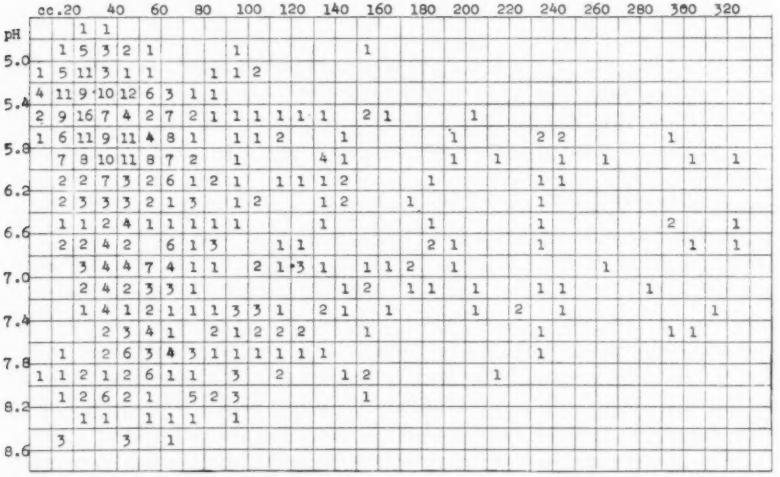

Fig. 1

no essential relationship between the two factors, except that when volumes were very small the acidity was higher than in others. The reactions of the specimens with larger volumes tended to be more alkaline, but there were enough specimens with large volumes and markedly acid reactions to show that such a relationship was not a necessary one.

In figure 3 the findings on some particular cases are represented graphically. Each line represents a case from which 3 to 6 (in most instances 5) specimens were studied. The length of the line represents the difference between the maximum and minimum acidity, and the vertical position of the line represents the reactions expressed in terms of pH. The cases have been arranged in order of the maximum difference in volume between any two specimens, and all cases which showed differences in volume of less than 50 cc. per hour are given. The figures below each

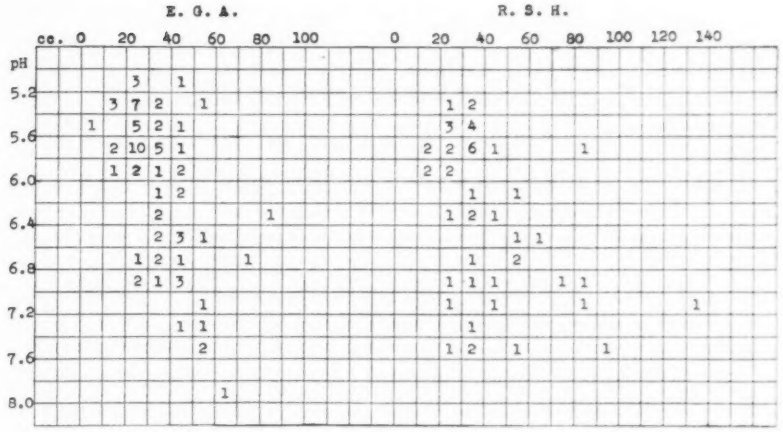
E. G. A.
R. S. H.
cc. 0 20 40 60 80 100 0 20 40 60 80 100 120 140
pH
5.2
5.6
6.0
6.4
6.8
7.2
7.6
8.0

Fig. 2

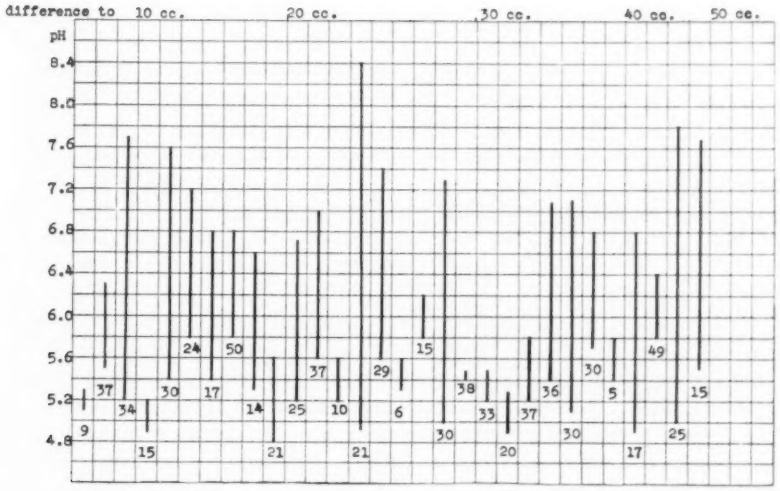
difference to 10 cc. 20 cc. 30 cc. 40 cc. 50 cc.
pH
8.4
8.0
7.6
7.2
6.8
6.4
6.0
5.6
5.2
4.8

Fig. 3

line record the number of cubic centimeters of the smallest specimen in that experiment. The chart shows that often where there was little difference between the volumes of different specimens from a subject there were marked differences in the reactions. There was possibly a tendency for small volumes and small differences in volume to coincide with distinctly acid reactions and relatively small differences in reaction, but the tendency certainly was not a marked one.

A chart similar to figure 3 which showed the cases with a small difference in the reaction of specimens was also prepared, but is not given. When studied in this way there was also little evidence of a relationship between differences in volume and reaction of specimens of urine collected from a patient. One subject who showed a difference in reaction of 0.1 pH showed a difference between the volumes of the specimens of more than 110 cc. per hour; another with a maximum difference in reaction of only 0.3 pH showed a difference of 250 cc.; and the subject with the smallest difference in volume—10 cc. per hour—showed a difference in reaction of 0.8 pH. There was possibly a tendency for small differences in volume and low values to coincide with small differences in reaction and higher degrees of acidity, but the tendency was certainly a slight one, and there were some striking instances in which there was no such relationship.

In general no relationship was found between the volumes and reactions of urine specimens excreted in an hour. When volumes were very small the acidity of the urine was usually high, but there were a number of instances in which this was not the case, and it does not seem justifiable to state that specimens with very small volumes formed an exception to the general rule. The relationship between the volume and reactions of specimens of urine which was shown in a statistical analysis of data reported in earlier papers must have been an incidental and not an essential one.

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THE EFFECT OF SLEEP ON BLOOD PRESSURE

M. A. BLANKENHORN AND H. E. CAMPBELL

From the Medical Department of Western Reserve University and of Lakeside Hospital, Cleveland, Ohio

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In the process of studying high blood pressure, one of us (Blankenhorn, 1921) devised a machine for the automatic recording of hourly blood-pressure determinations. Properly to evaluate the records of abnormal patients, a series of determinations was made on normal young adults. This shows a fall of pressure with the onset of sleep, most rapid in the first three hours, reaching the minimum pressure at the fourth hour, and a slight elevation in the two hours before waking. Within an hour after waking there is an abrupt rise to a point equal to the first hour of sleep and somewhat lower than the last waking hour (see fig. 1). This is contrary to the prevalent impression that the minimum is reached in the first two hours of sleep, and that the pressure rises gradually throughout the remainder of the night to a higher point on waking than existed the night before. We believe this impression prevailed because of the study of too few cases, the use of inaccurate criteria, the observation of unnatural sleep, or the lack of proper control of the hydrostatic factor involved when the patients sleep upon the side.

Leonard Hill (1898) was among the first to measure the blood pressure of man during sleep. He used the Hill and Barnard sphygmomanometer, recording mean arterial pressure as represented by the maximum oscillation of the needle; but McWilliams and Melvin (1914) and Erlanger (1916) have demonstrated that the maximum oscillation bears no constant relation to the mean arterial pressure, and that it varies with the state of contraction or relaxation of the arterial wall and with the volume of the compression chamber. Hill stated that rest and warmth induce a fall of pressure, and that the transition from waking to sleeping under these circumstances is not attended by further fall. He found the pressure as low when lying awake in the morning as when lying drowsy in the evening. A year or two later Brush and Fayerweather (1901) studied the blood pressure during sleep by means of the hand plethysmograph, likewise recording the mean arterial pressure as indicated by the maximum oscillation. They demonstrated a drop in pressure one to two hours after the onset of sleep, with a gradual rise in pressure during the night to a higher point on waking

than existed before sleep occurred, and noted a greater fall with sleep than with rest without sleep. Weyse and Lutz (1915) have shown the daytime variations.

The results of these authors may be combined to show a twenty-four-hour curve. From the values of Weyse and Lutz a mean pressure curve is constructed, on the principle propounded by Dawson (1906) and Erlanger (1904) that the mean arterial pressure is best represented by the diastolic pressure plus one-third the pulse pressure and not by the arithmetical mean of the systolic and diastolic pressures, since the pulse wave is not a truly triangular figure. An average of Brush and Fayerweather's "mean pressure" curves is taken, and assuming that the pulse pressure remains constant, the systolic and diastolic values are estimated by placing the systolic value at two-thirds the pulse pressure above, and the diastolic at one-third the pulse pressure below, the respective average mean-pressure values (fig. 1).

Brooks and Carroll (1912) also noted that the maximum fall occurred within one to two hours after falling asleep, and in sixty-eight cases of medium pressure noted the following systolic pressure variations:

4 p.m.	1–2 hours after sleep	3 hours after waking
142.5	118.5	130.5

Rather recent work has been done by Carl Müller (1921), who used a combination of the Riva Rocci and the Hill and Barnard instruments, and considered the first palpable radial pulse as the systolic pressure, and the widest excursion of the needle as the diastolic pressure. He made "numerous" blood pressure readings during sleep in thirty-four men and thirty women. The sleep was induced by small doses (0.5 gram) of veronal, but he found that such doses did not change the pressure more than a negligible 2 mm. The normal day-pressure varied from 110 to 130 mm., while the average minimum systolic night-pressure for men was 94 mm. and for women 88 mm., reaching this minimum within two hours after sleep began. Blume (1923), in a study of fifty patients, using the same technique, obtained similar results, 95 mm. for men and 88 mm. for women. These last two writers do not mention whether the readings were taken with the patient always on the back, or whether any correction was made for the hydrostatic pressure changes in case the patient was on the side when the reading was taken.

Our observations were made on twenty-five normal young men, with records for thirty-eight nights, the sleep being in no way induced. All but two of the men were patients on the wards of Lakeside Hospital, were ambulatory after their, as a rule, mild illness, were kept in bed throughout the experiment, were fed, but otherwise could be considered as observed

under basal conditions. Depending upon the wakefulness of the patient, we obtained from two to eleven consecutive observations definitely made on a sleeping subject. The sleeping or waking was estimated by the nurse in charge of the experiment for the night. Inasmuch as it is at best difficult to decide whether or not a man is asleep without actually wakening him, the nurse was required to do no more than record the position of the patient and her opinion as to whether or not he had been awakened by the machine.

It was also possible to obtain a record of the pulse rate with the apparatus. If the pulse was counted at the same time a tracing was being made, the pulse rate at each subsequent tracing could be computed, it being assumed that the volume of the cuff and the rate of escape were constant. Since the cuff was of inelastic material, was fitted quite snugly and was found not to slip, and since the rate of escape could not vary, this method was considered fairly accurate. Unfortunately, the pulse was not always counted at the moment the tracing was being made; hence the rate computed from the tracings was not always accurate, although the relative variation from hour to hour could be so considered. The pulse rates on the chart represent the averages of the values obtained by this method.

We found that the changes upon the pressure reading caused by the patients' lying upon the side could be explained by the hydraulics involved. If the arm bearing the cuff was lying along the uppermost side, the pressure reading was reduced by the mercurial equivalent of the distance from the midline to the lateral chest wall. If the arm was allowed to fall across the front of the chest, the reduction of the reading was considerably less in some cases, in others almost nil, since the cuff was at these times almost on the level of the heart. If the patient lay on the side bearing the cuff, the reading was increased somewhat, although not nearly to the same degree that it was decreased by lying on the other side. This depended upon the location of the arm, whether it was under the body or whether it was brought forward. We made numerous observations on ten patients and found an average change in pressure reading as follows:

Cuff on left arm

PATIENT ON RIGHT SIDE		PATIENT ON LEFT SIDE	
Systolic	Diastolic	Systolic	Diastolic
−11.2 mm.	−9.8 mm.	+1.0 mm.	+2.0 mm.

The average thoracic diameter of these ten subjects was 28.6 cm., which agrees closely with the average of 29.02 cm. obtained from 96,000 white troops. Hence it is seen that the change in pressure reading when the patient is lying on the right side with the cuff on the left arm can be ex-

plained by the hydrostatic change, i.e., 10.5 (the mean of 11.2 and 9.8) $\times$ 13.65 (the specific gravity of mercury) = 14.2; and one-half the thoracic diameter = 14.3 cm. The reason that the reading made with the patient on the left side is not correspondingly increased seems to be that the arm is crowded around to the front of the chest so that the middle portion of the brachial artery is found almost on the level with the sternum.

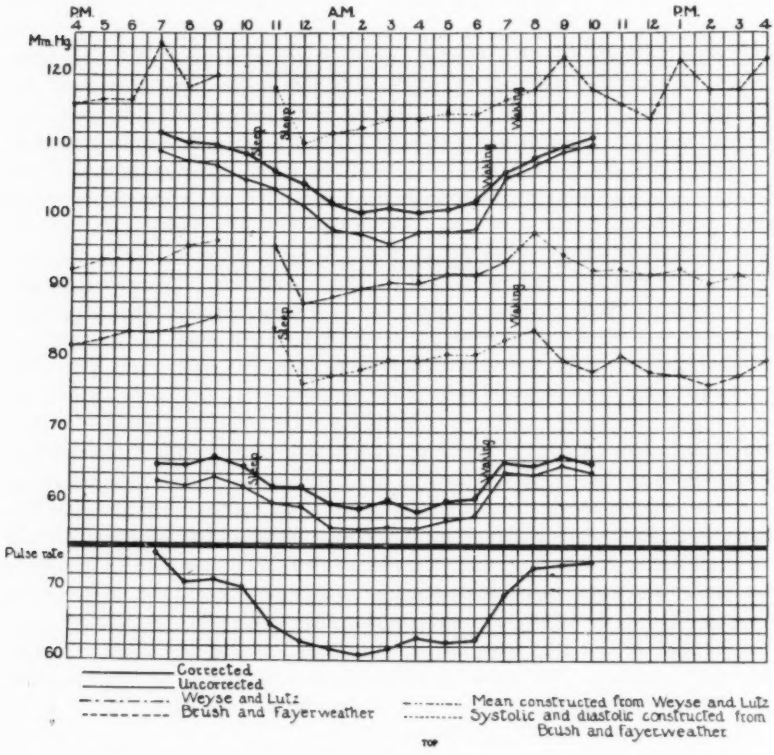

Fig. 1. Average hourly pressure readings of 25 normal young men, with *1*, correction for lateral position during recording, and *2*, comparison with previous conceptions of the sleeping pressure.

It was found by examining several patients during sleep that the uppermost arm was most commonly allowed to lie along the side, the forearm being flexed or extended at random. Since most of the subjects chose to sleep on the right side, we placed the cuff on the left arm and made the observation each hour of the day and night as to the position assumed. If the patient was lying on the side, we assumed that the arm lay along the

lateral thoracic wall, and did not attempt to determine whether it was across the chest for fear of waking him. On reading the tracings, therefore, those that were made with the patient lying otherwise than on the back were corrected by the average correction given above.

We made determinations upon thirty-eight young men, but present only twenty-five because the others had some evidence of nephritis, or slight temperature elevation with increased pulse rate, or did not tolerate the apparatus. Hence our twenty-five cases are as near the normal as could well be found among hospital patients. The number of consecutive hours of sleep ranged from two to eleven, with an average of five, and the total hours of sleep each night was from four to eleven, with an average of seven and a half (fig. 1).

Discussion. It has been stated by previous workers that the minimum pressure is reached in the first two hours of sleep. This we believe is due to the faulty method of taking the blood pressure, or to the induction of sleep by drugs. It seems quite probable that, in even mildly induced sleep, particularly in that induced by veronal, which produces vasodilatation, the fall to the minimum would occur earlier than in natural sleep. Also it seems that the pressure readings were not taken regularly throughout the night, and hence the low point was probably not observed. Our results seem to show that this fall is a more gradual process and that the minimum is reached between the third and fourth hours of sleep.

It is interesting that our uncorrected average for the lowest pressure reached during the night is so nearly that obtained by Müller and by Blume. Our uncorrected reading is 96.3 mm. and theirs 95 and 94, respectively. These authors make no mention of applying a correction for the turning on the side. However, if the turning on the side is taken into consideration, we find the average minimum systolic pressure to be 101 mm. As seen in figure 1, this point was reached at the fourth hour of sleep and it continued with 0.4 mm. fluctuation for four hours.

It is to be noted that the minimum systolic pressure is but 11 mm. lower than the average highest day pressure. It must be borne in mind that our subjects were purposely kept in bed throughout the day so as to maintain as nearly as possible rest or "basal" conditions. The day pressure is therefore considerably lower than if they had been active. Perhaps the average highest day pressure figure would be higher if the readings were grouped in relation to meal times, since Weyse and Lutz demonstrated a definite elevation following meals. We regret that we cannot present a complete twenty-four-hour curve, for while we appreciated its desirability, we were unable to control all the factors necessary to its attainment.

We also present a diastolic curve, and its low plane is immediately striking. This is undoubtedly due, in even greater part than in the systolic

curve, to the rest or "basal" conditions obtaining throughout the experiment.

The pulse rate is seen to reach the lowest point at the fourth hour of sleep, and it would seem probable that the fall of blood pressure is the direct result of the fall of pulse rate and only secondarily the result of sleep.

SUMMARY

1. We have described an apparatus for the automatic recording of serial blood pressure determinations.

2. The tracings obtained concur with the accepted idea of the oscillatory criteria.

3. Hourly blood-pressure determinations for the greater part of the twenty-four-hour period under "basal" conditions are presented, the sleep being as natural as possible and the only definite variable in the experiment.

4. The averages of twenty-five patients and thirty-eight nights are offered, showing the occurrence of the minimum systolic point (101 mm.) at the fourth hour of sleep, a slight rise before waking, and an abrupt rise after waking to a point equal to the first hour of sleep. The diastolic pressure shows a like though lesser fall.

5. The pulse shows a curve almost similar to that of the pressure.

6. The fall of the pressure is owing mainly to the fall of pulse rate, though it is probably somewhat influenced by the peripheral relaxation.

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CONDUCTIVITY IN COMPRESSED CARDIAC MUSCLE[1]

I. The Recovery of Conductivity Following Impulse Transmission in Compressed Auricular Muscle of the Turtle Heart

RICHARD ASHMAN

From the Physiological Laboratory of the Tulane University of Louisiana, School of Medicine

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A fuller understanding of heart-block and of the effects which variations in the conditions of cardiac activity have upon it requires a knowledge of conductivity in cardiac tissue which at present is not available. Measurements of the duration of the conduction times following the arrival of impulses at a compressed region in the auricular muscle of the turtle's heart are reported in this paper with a view to answering the following questions: What is the course of recovery of conductivity in the compressed muscle following the transmission of an impulse through that region? What is the effect of a blocked impulse upon conductivity?

It has been well known ever since the early investigations of Gaskell (1) and of Engelmann (2) that an impulse *transmitted* through the junctional tissue between auricles and ventricle in the cold-blooded heart reduces the conductivity so that a second impulse, arriving early, is either blocked or transmitted more slowly than through the resting tissue. That a *blocked* impulse, although failing to elicit a ventricular response, does nevertheless lengthen the conduction time for a subsequently transmitted impulse is not so well established, although it is recognized that such impulses must have a definite influence. This fact is brought out clearly in the work of Garrey (3), of Erlanger (4) and of Lewis (5). Garrey calls attention to the fact that a slowing of the auricular rate may permit all impulses to pass a compressed region in the auricular muscle of the turtle heart when previously a high grade or even complete block was present. Erlanger, and also Lewis, have pointed out that in the mammal, or in man the syncopal attack of Stokes-Adams syndrome, due to ventricular stand, still, may be precipitated by a sudden acceleration in the auricular rate.

In contrast to this recognition of the effect of blocked impulses is the view ordinarily implied, if not expressed, that in cases of partial heart-block the blocked impulse produces no effect and that consequently there

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is recovery of conductivity so that the next impulse succeeds in passing the injured region.

In brief, the fact that with impaired conduction, a sudden increase in the frequency with which the impulses arrive at the block may cause a ventricular standstill proves that it is incorrect to assume that they produce no effect. On the other hand, it is evident that their influence cannot be as great as that of transmitted impulses, for otherwise a 2:1 block would immediately become complete. Such considerations afforded the starting point for our investigations.

The preparation which seemed best adapted to this study of conductivity was the excised turtle heart in which the conductivity was locally reduced by compression with a Gaskell clamp. The impairment of conductivity due to compression is similar to that produced by a local increase in the hydrogen ion concentration. For an understanding of the conditions prevailing in our experiments the following brief survey of studies of conductivity made under conditions which may be regarded as leading to an increase in the hydrogen ion concentration will suffice. That injury to tissue will produce a local electronegativity has long been known. Prof. W. E. Garrey states (verbal communication) that compression with a clamp has a similar effect. The velocity with which the cardiac impulse is transmitted has been shown to decrease under conditions which may be regarded as causing an increase in the cH within the tissue, i.e., with fatigue, lack of oxygen, injury or perfusion with acid or neutral solutions. Thus Engelmann (2), and later Skramlik (6), reported that the A-V interval gradually becomes longer in the excised, dying frog heart; and Engelmann also found that a rapid series of auricular impulses was followed by a prolongation of the A-V interval. Lewis and White (7) demonstrated an increase in this interval in the cat's heart due to asphyxiation; while Drury and Andrus (8) have recently shown that conduction through the auricular musculature of the dog's heart is slowed both by lack of oxygen and by an increase in the hydrogen ion concentration of the perfusate. Drury (9) reported slowing of conduction of the impulse through the compressed auricular muscle of the dog's heart, and also conduction with a decrement. Mines (10) found an increase in the A-V interval resulting from an increase in the cH of the perfusate in the atropinized frog heart. Gaskell (1) noted the marked prolongation of the conduction time between parts of the turtle heart separated by a clamp or by cutting so that only a narrow connecting bridge remained. Investigations employing such artificial blocks have repeatedly been made since, e.g., by Erlanger (11), by Garrey (3) and by Lewis and Drury (12). Further, in clinical cases of partial heart-block it is a common observation that the A-V interval is increased as a result of injury to the conducting pathway between auricles and ventricles.

Although the above investigations were all made under conditions in some respects comparable to those obtaining in our experiments, they do not give information as to the course of recovery of conductivity in the cardiac muscle, information which we have obtained. Curves which may be regarded as representing the course of recovery of conductivity have been figured by Mines (13), (14) for the atropinized, but uncompressed frog heart; and similar curves might be constructed from certain tables given by Engelmann (2); but we are unable to find similar analyses for tissues which have been compressed locally, and in which, therefore, the hydrogen ion concentration may be regarded as having been increased. Mines determined the A-V intervals by means of the string galvanometer when the auricles of the quiescent frog heart were stimulated at various rates. Since no artificial block was established the curves are directly comparable to those shown in our figures 4 *D* and *E*, and 5 *D* and E. Mines avoided the term refractory phase when speaking of the curves representing the variations in the A-V intervals, and apparently did not think of his curves as representing the course of recovery of conductivity in the junctional tissue. He attributed the variations in the interval to changes in the hydrogen ion concentration consequent upon the increased metabolic activity with the higher cardiac rate and believed that for each rate a fixed cH was established.

The present investigation was undertaken from a different viewpoint. The conditions of the experiments were different in that a block was established and thus, it is believed, the *variations* in the A-V intervals are in many cases due almost entirely to variations in the conditions within the compressed muscle. Whether the recovery curve represents the disappearance of a refractory phase or whether it reflects merely a change in the hydrogen ion concentration is left undecided. As has already been stated, in addition to tracing the course of the recovery of conductivity, the effect upon conductivity of blocked impulses is studied. So far as the writer is aware this has not hitherto been attempted.

THE METHOD. The experimental animals were turtles of the species *Chelydra serpentina* and *Chrysemys elegans* and *troosti*. Atropin sulphate (2 or 3 drops of a 0.1 per cent solution) was mixed thoroughly with the pericardial fluid to prevent vagus effects due to direct electrical stimulation of the auricles. As soon as vagus stimulation indicated complete paralysis of the vago-inhibitory endings, the entire heart was removed from the animal. The sinus venosus was cut away and this usually caused cessation of spontaneous beats. Unless automatic beats were spaced at intervals of 15 seconds or more, the hearts were discarded. The heart was placed, ventricle uppermost, in a modified heart clamp, open at one side. The jaws were covered with pure gum tubing so that compression of the muscle could be brought to the desired stage of block without danger

of permanent injury. Arrangements were made to record, with light heart levers, the systoles of one auricle, usually the right, and of the ventricle.

The compression is believed actually to have been applied to auricular muscle, the clamp being placed in the auriculo-ventricular groove trans-

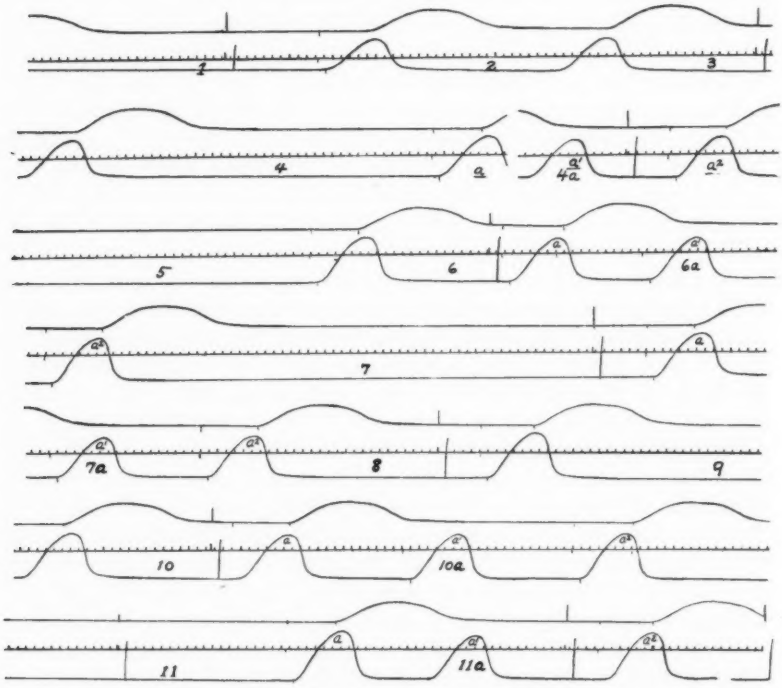

Fig. 1. Series 6, January 23, 1925. Myograms from turtle heart in which, by means of a clamp, compression had been established in the auriculo-ventricular groove. Auricular contractions below. Stimulation, electrical. The series is continuous, excepting that 1.4 seconds is omitted between *4* and *4a*, 4.0 seconds between *4a* and *5*, and 3.2 seconds between *9* and *10*. The tracings are retraced and bleached prints of the original myograms. Time in fifths. Temperature 25°C.

versely across the heart. The amount of compression necessarily varied at different times, but was usually such as to give a partial block, e.g., 2:1 when the auricle was stimulated at a moderate rate. Contractions were produced in the quiescent auricle by stimulating with break induction shocks, the makes being subminimal and not short-circuited. The stimulating current was led from the secondary of a Harvard inductorium by means of two metallic tinsel conductors attached to wire serrafines

which were placed on the auricle as near each other as practicable but not near the compressed tissue, thus preventing spread of current to the region of compression. Tracings were obtained with time recorded in fifths of a second by a Jacquet chronograph, the kymograph being driven at the speed illustrated in figure 1.

A series of contractions extending in most cases the length of the long-roll kymograph paper constituted a unit in the experiment. The auricle was stimulated at time intervals varying with temperature and the degree of compression employed from a few up to 12 to 16 seconds. In this way A-V intervals of various durations, depending upon the length of the preceding rest period, were obtained. An automatic regulation of the intervals between stimuli was not attempted, nor would it have been practicable since the frequency of stimulation had to be regulated and varied in accordance with the conditon of the tissue existing when each series was begun. The stimuli were thrown in at irregular intervals as may best be seen from figure 1, with longer intervals (10 to 16 seconds) here and there in the series to minimize, as far as possible, fatigue effects as well as to determine the A-V interval for the resting heart. In many of the hearts a normal or control series was obtained before pressure was applied to the muscle. From measurements of each series obtained a curve was constructed. Since it is not possible to keep the physiological state of the compressed tissue constant over a long period it is necessary to construct each curve from a single series which has been recorded within two or three minutes.

In many of the series the interval between auricular contractions was occasionally so short that the second impulse was blocked. Where several such blocked impulses occur in a single series it becomes possible to compare the effect of the blocked and of the transmitted impulses upon subsequent conduction time.

The analyses are based upon about 70 measured series obtained from 22 hearts. This is exclusive of the considerable number of series which showed a supernormal phase to be discussed in the second paper of this series. The effect of approximately 160 blocked impulses has been determined and their effect upon conductivity compared with the effect of the impulses which passed through the compressed muscle.

RESULTS AND DISCUSSION *A. The recovery of conductivity in compressed auricular muscle subsequent to the passage of an impulse.* The A-V interval is taken as an index of the conductivity. In general this interval is shortest when there has been a long rest between the auricular contractions. As this period of rest becomes shorter, providing it does not permit complete recovery, the A-V interval increases. Typical examples of the effect of such variation in auricular rate, i.e., in the rate at which impulses arrive at the compressed tissue, upon the A-V interval,

are shown in the following table and figures which represent the course of recovery of conductivity in the compressed muscle.

Table 1 gives measurements of a single series from which figure 1 has been constructed. The numbers in the first column are for reference to individual auriculo-ventricular pairs of contractions or complexes. In the second column the ratio between the number of auricular systoles and the succeeding ventricular beat is given. Thus, if, as in complex 4a, a single auricular impulse was blocked while the ventricle responded to the second, we have a 2:1 relation. In the third column the intervals between the auricular beats are indicated in seconds. In case of a 2:1 block, both interauricular intervals are noted with a plus sign between and the sum of the two follows in parenthesis. In the fourth column, the

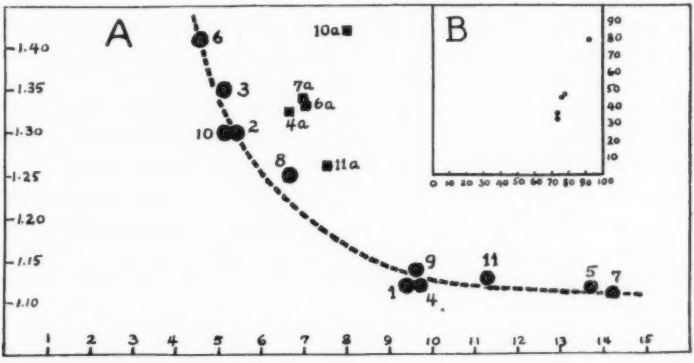

Fig. 2. *A*, series 6 of January 23, 1925. Temperature, 25°C. From measurements given in table 1. Ordinates represent the A-V intervals in seconds; abscissae, the intervals between auricular systoles in seconds. Each square represents the A-V interval following a 2:1 block. Note that, owing to the interpolation of the blocked impulse, the points do not fall on the curve.

B, the effect of blocked impulses in figure 6*A*. For explanation see text and figure 6*B*.

auriculo-ventricular (A-V) intervals are given in seconds. This will be clear if reference is made to figure 1 in which the interauricular intervals have been numbered to correspond to the table. From measurements given in table 1 the curve (fig. 2) is constructed. In the construction of this curve, the interauricular intervals are plotted as abscissae and the consequent A-V intervals as ordinates. Such a curve represents the actual ability of the compressed muscle to conduct impulses at any instant following the passage of an impulse through that tissue. Some seventy similar curves have been constructed from data which are available for the analysis of our experiments. (Those points represented as solid squares are not related to the recovery curve and are discussed below.)

An analysis of the results of our experiments must take account of the fact that each A-V interval has at least three components: the time required for the impulse *1*, to traverse the auricular muscle from the point stimulated to the compressed region; *2*, to pass through the compressed muscle, and *3*, to traverse the tissue beyond the compressed region and

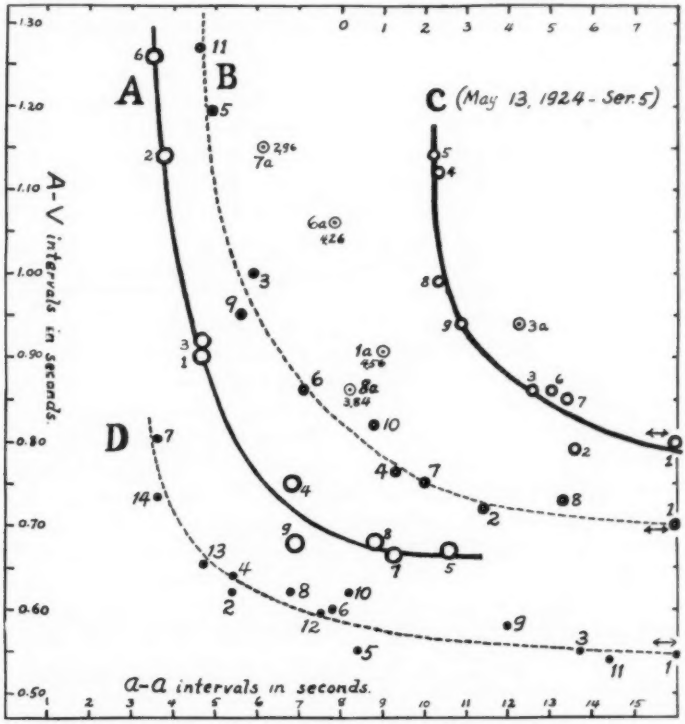

Fig. 3. *A*, from series 1 of January 23, 1925. From this curve are omitted 2:1 blocks occurring after points *2*, *3* and *4*; and a 4:1 block after point *8*. The first point recorded was omitted since the interauricular interval was unknown. *B*, series 2, January 23. A 3:1 block after point *2* is omitted. The arrow under point *1* means that the interauricular interval is not known, except that it was relatively long. Temperature, 25°C. *C*, abscissae for this series above. *D*, series 1 of January 10, 1925. Blocks after points *1*, *4*, *7* and *14* omitted. Temperature, 28°C.

the junctional tissue. If it be assumed that the interval between the electrical and mechanical response in the auricle is different from that in the ventricle, another component may be added (or subtracted). The *variations* in the A-V intervals which depend upon the duration of the interauricular or rest period (i.e., those variations which give the curve

of recovery of conductivity), may be due to variations in the time required for the impulse to travel through any or all of the regions enumerated. But if it can be shown from the time relations that the recovery of conductivity in all parts of the heart other than that compressed at the time each A-V interval of a series is being recorded, then the *variations* in the A-V interval must be due entirely to changes in conductivity within the

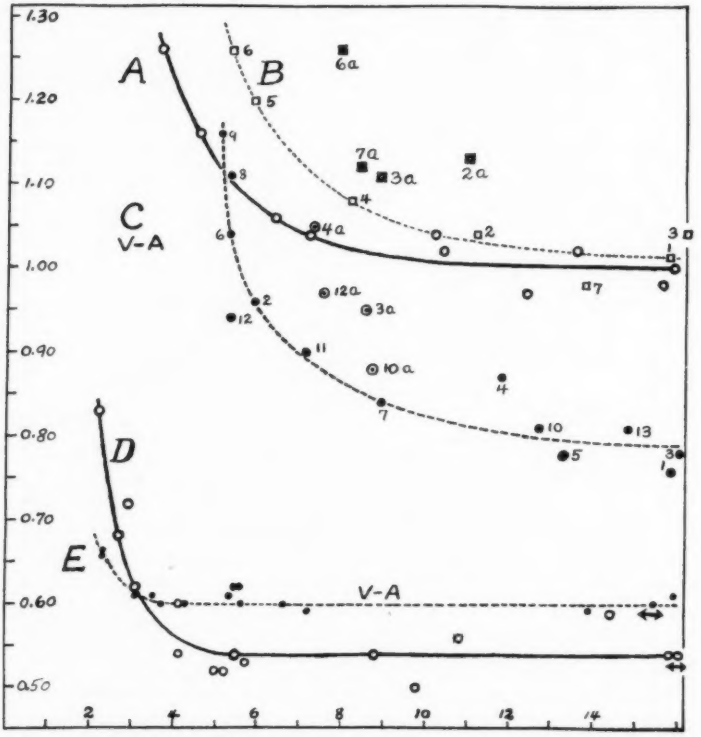

Fig. 4. All of February 4, 1925. *A*, series 5. *B*, series 10. *C*, series 11. *D*, series 1, *E*, series 2. The series are numbered in sequence as they were recorded. Abscissae and ordinates as in figure 2. Descriptions in text. Temperature, 25°C.

compressed muscle. This can unquestionably be shown for a few of our series, namely, those in which the interval following the passage of one impulse, during which a second impulse would be blocked, was sufficiently long; and which further were obtained early during the course of the experiment and hence before much fatigue was induced. Evidence for this statement can be had from a comparison of figure 4 *B* and *D*, which

shows that by the time the first impulse was able to pass in curve *B* (5.3 seconds after previous impulse transmission), *D*, which represents the recovery in the uncompressed heart, had fully recovered. This can only mean that the differences in A-V interval shown at various times along curve *B* are due to the state of conductivity in the compressed muscle and in that muscle only; for, by the time recovery of conductivity is sufficient to allow a second impulse to pass, the conductivity in all other parts of the heart, excepting that compressed, will have completely recovered as is shown by curve *D*. Even clearer evidence in support of this conclusion is to be found in figure 4 *C* and *E*, although in this instance conduction was from ventricle to auricle. Equally conclusive evidence of the same kind is found in experimental results which it seems superfluous to publish in detail. In some instances an objection may be raised on the basis of work reported by Engelmann (2) and by Skramlik (6) who found that in the dying frog heart the A-V interval gradually increases. Since a variable period elapsed between the recording of the control series and of the later series in our experiments it must be admitted that the condition of the tissue outside of the compressed region, particularly of the junctional tissue between auricles and ventricle, may have changed enough to have contributed in slight measure to the variations obtained; but this is not the case in series obtained early which yield similar results.

One other piece of evidence may be advanced to show that it is possible to obtain curves showing almost exclusively the recovery of conductivity in the compressed muscle. In brief, this lies in the effect of the blocked impulse which is discussed in detail below. The blocked impulse may have a very great effect upon the A-V interval for a subsequent transmitted impulse, and it has this effect in spite of its failure to reach the junctional tissue in which undoubtedly the greatest variations in the A-V interval, aside from those in the compressed muscle, are to be sought. If the blocked impulse can have so great an effect in spite of its failure to reach junctional tissue it seems improbable that that tissue can play much part in causing the variations in the A-V interval for the transmitted impulse.

It thus becomes possible to analyze the course of recovery of conductivity in the compressed muscle with the assurance that variations in the A-V interval due to the uncompressed tissue are negligible.

It may be concluded from such analysis of our results that the conductivity in the compressed muscle, following the passage of an impulse through that muscle, is at first so depressed that a subsequent impulse fails to pass to the ventricle. After a period an impulse is able to pass, but it travels slowly. From this time the recovery of conductivity is at first rapid, then more and more slow until complete recovery has occurred and the shortest A-V interval is obtained. The curves of re-

covery of conductivity are strikingly like those obtained by Adrian (15) for the recovery of excitability in both nerve and the ventricular muscle of the frog; and by Trendelenburg (16) for the excitability of both the auricle and the ventricle of the frog. Our curves of conductivity for the heart in which no compression has been established (fig. 4 *D* and *E*; fig. 5 *D* and *E*) are like those found by Mines (13), (14) for the atropinized frog heart; and similar curves could be drawn from certain tables published by Engelmann (2).

It would be premature to attempt to decide whether the curves represent the disappearance of a relative refractory period in the compressed muscle, a period more or less independent of the hydrogen ion concentration, or whether they reflect the rate at which the hydrogen ion concentration increase, due to the activity of the muscle, is reduced, as Mines (13) seems to have believed. One fact should be emphasized, i.e., that if the impulse is conducted with a decrement in the compressed muscle, as Drury (9) has reported, then that interval during which a second impulse is blocked is of greater duration than the true absolute refractory period, and the recovery curve does not show the whole of the relative refractory period. That period must already have begun before an impulse, conducted with a decrement, could pass.

Reverse conduction. While the above conclusions were reached in regard to conduction in the normal direction, attention should be called to the fact that they apply equally to conduction in the reverse direction. In illustration of this figure 4, *C* and *E*, is given. The curve *C* is similar in every way, including the effect of blocked impulses discussed below, to a curve representing A-V conduction.

A further point which may be of interest is brought out by a comparison of figure 4 *D* and *E*. These curves are from the same heart to which no compression had been applied, although a conducting bridge of auricular tissue was somewhat narrowed when the sinus was cut away. Although the resting A-V interval is shorter, *D*, than in the reverse direction, *E*, the recovery of conductivity is not so prompt. An insufficient number of A-V series, without block, have been obtained to make it possible to say whether this relation is at all general. Whether this difference can be taken as evidence for the view advanced by Skramlik (6) that forward and backward conduction are easier along different pathways must be decided by future work.

Fatigue effects. Changes which may tentatively be spoken of as "fatigue effects" may be noted in several of the curves. A clear example of such a phenomenon is to be seen in figure 5 *B*. If the reader will keep in mind the fact that the individual points are numbered in the sequence in which they were recorded on the tracing, and that each lettered block (circles) follows the point of similar number, it will be seen that the points

tend to fall above the line if they have been preceded by much *recent* activity. Thus there is indicated a cumulative "fatigue effect" which leads to a prolongation of the A-V interval. Point *3* in this curve, which is preceded by eight auricular contractions in rapid sequence, is the only exception. Figure 5 *C* is another example of the influence of fatigue. Here point *8* seems to be the only exception.

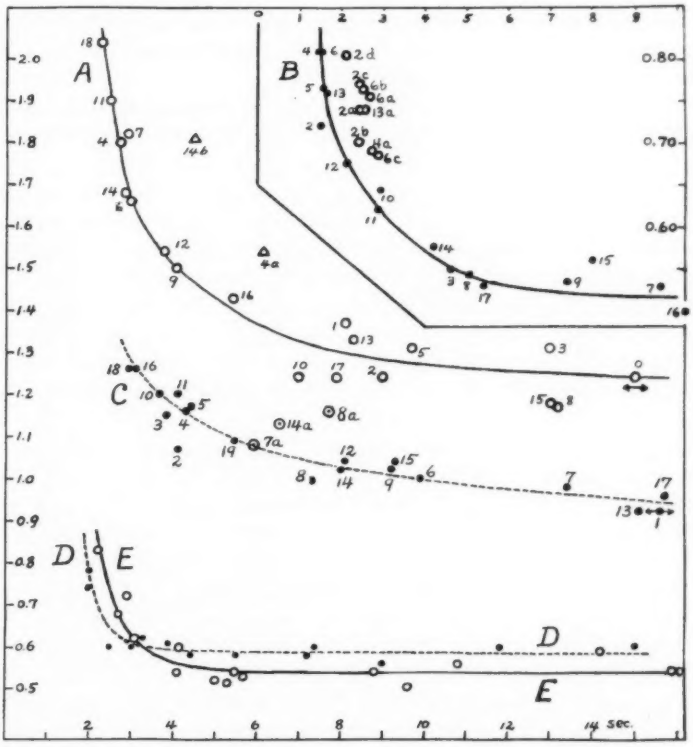

Fig. 5. *A*, series 9, January 23, 1925. *B*, series 3, June 6, 1924. *C*, series 5, January 29, 1925. *D*, series 1, February 2, 1925. *E*, series 1, February 4, 1924. Plotted similarly to figure 2. Descriptions in text.

Over half of our series show such fatigue effects more or less clearly. On the other hand no such effects are to be detected in figure 5 *A* nor in any of the other curves from this heart (fig. 2, fig. 3 *A* and *B*). It may be that these fatigue effects are caused by the more rapid production than removal of acid products in the compressed muscle; while the recovery from fatigue after longer rest periods (see points *16* and *17*, fig.

5 *B*) may mean that the removal is more rapid than the production. The writer believes that such effects may have an important bearing upon the question of partial heart-block.

Tonus and conductivity. It may be stated that no relation has been found between the A-V interval and tonus waves in the auricle. Prof. W. E. Garrey has noted this same lack of influence of tonus waves upon the A-V intervals in electrocardiograms from the turtle heart. Porter (17) reported that conductivity in the auricle was decreased by tonic contractions. Apparently what he actually determined was a change in the latency to electrical stimulation.

B. The influence of blocked impulses upon the conductivity of compressed auricular muscle. One of the important objectives of this investigation

TABLE 1

Series no. 6, January 23, 1925

NUMBER	DEGREE OF BLOCK	TIME IN SECONDS BETWEEN AURICULAR BEATS	A-V INTERVAL IN SECONDS
1	1:1	9.20	1.12
2	1:1	5.40	1.30
3	1:1	5.16	1.345
4	1:1	9.68	1.12
4a	2:1	3.20+3.42 (6.62)	1.325
5	1:1	13.66	1.12
6	1:1	4.56	1.41
6a	2:1	3.34+3.68 (7.02)	1.33
7	1:1	14.20	1.11
7a	2:1	3.42+3.56 (6.98)	1.34
8	1:1	6.62	1.25
9	1:1	9.60	1.14
10	1:1	5.14	1.30
10a	2:1	4.04+3.96 (8.00)	1.42
11	1:1	11.28	1.13
11a	2:1	3.20+4.16 (7.36)	1.26

was a quantitative determination of the effect of a blocked impulse upon the A-V interval for a subsequently transmitted impulse. In order to measure this effect it is obviously necessary to know just what the A-V interval would have been had the blocked impulse not been interpolated. This information was obtained by securing a number of 2:1 blocks during the course of a series, such as figure 1 (table 1, fig. 2) from measurements of which a curve of the recovery of conductivity could also be constructed. It was soon found that the desired information could not be obtained merely by noting the absolute increase in the A-V interval caused by the blocked impulse, since the magnitude of the increase depended upon the interval which elapsed between the arrival at the compressed region of the blocked and of the subsequently transmitted im-

pulse. Consequently a method of determining the influence of the blocked impulse was employed which makes possible a direct comparison of the effect of blocked impulses arriving at various times during the interval when no impulse will pass, and of blocked impulses in different series. This comparison of the effects of blocked impulses of different series is desirable because of the limited number of blocks which can be obtained from a single series, a limitation due to the instability in the condition of the compressed muscle. At this point it is essential to consider in detail the analysis by which the effect of blocked impulses was determined.

Taking the ventricular contraction no. 6a in table 1, we find that two auricular contractions preceded it, the first of which was blocked, thus giving a 2:1 block. If we denominate the auricular contraction of the preceding complex, no. 6, a, the blocked impulse, a^1, and the next conducted impulse, a^2, we can represent their time relations as myograms and arrows as in figure 6 *A* in which the curve R is that of figure 2 on a different scale. By reference to figure 2 it will be seen that if impulse a^1 had not been interpolated, the recovery curve following impulse a would have taken the course R, and impulse a^2 would have had a conduction time of 1.20 second. The actual conduction time for a^2 was however 1.33 seconds, indicating that a^1 leaves its own impress upon conduction time. If we assume for the sake of convenience that the curve of recovery of conductivity following a^1 has the same form as R, we may then represent that recovery by R^1 which is so drawn that it pases through the point *6a* and that the horizontal distance between R and R^1 is constant. R^1 thus represents, on this assumption, the extent to which the recovery curve is displaced to the right by the blocked impulse at a^1. All experiments show that if a^1 had followed a at a shorter time interval, R^1 would have been displaced much less to the right; if a^1 had fallen later, the displacement to the right would have been greater. The experimental evidence on which this generalization is based is presented below. We first analyze a specific instance from a single series.

For each block in table 1, nos. 4a, 6a, 7a, 10a and 11a, the a-a^1 (interauricular) interval is calculated in terms of *percentage* of the a-R interval, i.e., that interval during which no impulse will pass (see fig. 6 *A* and also fig. 2); and the displacement of the curve to the right, the R-R^1 interval, is also stated in terms of percentage of the a-R interval. Thus, as may be seen from figure 2, for block 4a the a-R interval is about 4.40 seconds, the a-a^1 interval is 3.20 seconds or 73 per cent of the a-R, while the displacement of point 4a to the right of the recovery curve R, i.e., the R-R^1 interval, is about 1.54 seconds or 35 per cent of the a-R interval. Plotting the percentage values of a-a^1 and of R-R^1 against each other for all blocks in this series gives figure 2 *B* which therefore expresses graph-

ically the fact that impulses arriving at the compressed region early during the period when impulses do not pass (i.e., the a-R interval) have less effect upon the lengths of subsequent A-V intervals than impulses arriving later. Four other instances showing a similar effect of blocked impulses on conductivity in series obtained from other hearts are presented in figure 7A. The group of points from each series is represented just as in figure 2 *B*.

In order that there may be no doubt in the mind of the reader that in general the later a blocked impulse falls during the a-R interval the greater is its effect upon subsequent conductivity, figure 6 *B* is given

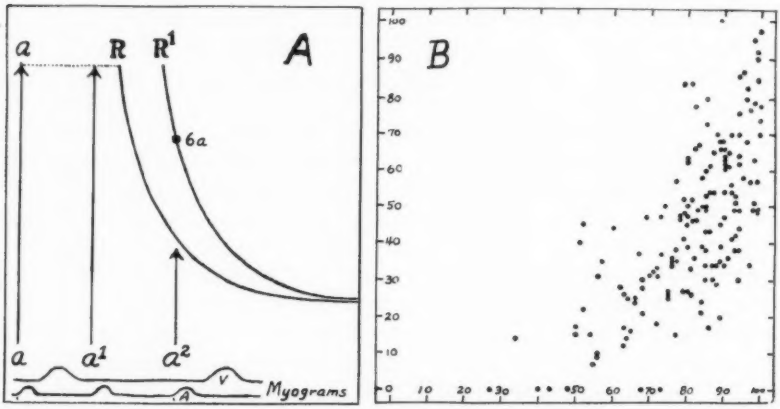

Fig. 6. *A*, see description in text. *B*, the effect of blocked impulses. Ordinates represent the R-R′ interval, i.e., the horizontal distance from the recovery curve to the point for the 2:1 block, expressed as percentage of the a-R interval, i.e., the interval during which no impulse will be transmitted to the ventricle. Abscissae represent the a-a^1 intervals, i.e., the interval from the beginning of the last conducted auricular systole to the beginning of the blocked auricular beat, expressed as percentage of the a-R interval. Thus the figure shows that the later a blocked impulse arrives at the compressed region, the greater is its effect upon the subsequent conduction time.

which includes the data on the effect of blocked impulses from all measured series excepting those showing a supernormal phase in conductivity in which case the relations are complicated, and those from series for which it was impossible to draw an accurate recovery curve. Certain individual points were also omitted because the interval following the blocked impulse was so long that the influence of that impulse was inappreciable. In this figure (6 *B*) the abscissae represent the a-a^1 intervals in terms of percentage of the a-R intervals; the ordinates represent the R-R^1 intervals also in percentage of the a-R intervals. For comparison it may be

stated that a transmitted impulse would have displaced the curve R to the right exactly the a-R distance, and therefore the value of a transmitted impulse, if represented in this figure, would have been 100 per cent. It will be seen from the grouping of the points that the earlier the blocked

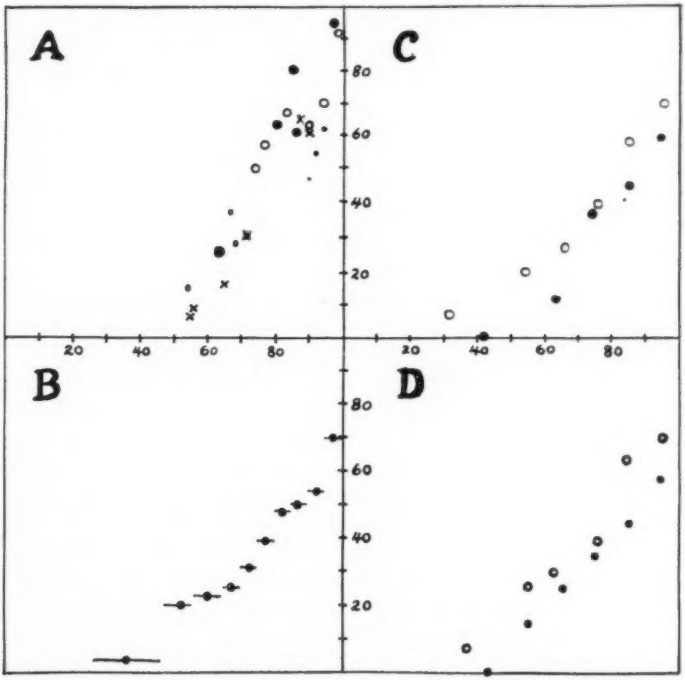

Fig. 7. *A*, same explanation as figure 6 *B*. Large black circles represent blocks from series 7 of May 17, 1924. Circles, from series 6 of the same date. Dots, from series 2 of January 12, 1925. *X*'s from series 3 of May 20, 1924. *B*, averages of all data on the effect of the blocked impulse. The horizontal lines through each point indicate the percentage range of abscissae values averaged for the point. *C*, circles, averages of all blocks from series with long a-R intervals. Solid circles, from series with short a-R intervals. The intermediate range omitted. *D*, circles represent averages of effect of blocked impulses in cases where the a^1-a^2 interval was above 95 per cent of the a-R interval. Solid circles, averages in cases where the a^1-a^2 interval was below 85 per cent of the a-R interval.

impulse follows a transmitted one, as represented by a small percentage value on the abscissae, the less is its effect upon the conduction time for a subsequently transmitted impulse, as shown by the correspondingly small percentage value on the ordinates; and conversely, that the later

the blocked impulse follows the transmitted one, as shown by a large percentage value on the abscissae, the greater is its effect upon conductivity, as indicated by a large percentage value on the ordinates.

This becomes still clearer when we plot points which represent the averages of all the points shown in figure 6 *B* and thus obtain the graph shown in figure 7 *B*. The horizontal lines drawn through the points represent the range in the percentage values of the a-a^1 intervals whose corresponding R-R^1 intervals, also expressed as percentage of the a-R intervals, have been averaged.

Relation between length of the a-R interval and the effect of blocked impulses. In an earlier part of this paper reference was made to the fact that where the a-R interval was long, the junctional tissue played no part in producing the variations in the lengths of the A-V intervals for those series, because in those cases the conductivity of all the cardiac tissue, other than that compressed, had completely recovered before any impulse could pass the compressed region. The impression must not be gained, however, that the junctional tissue can not, under certain conditions, play a distinct part in producing the variations shown in those curves where the a-R interval is short. In order to determine whether any difference could be brought to light by plotting separately the blocks in case of long and of short a-R intervals (due allowance being made for temperature differences) figure 7 *C* was constructed. Each point is an average of the number of individual points indicated by the accompanying number. The circles show the average effect of blocked impulses for all series in which the a-R interval clearly exceeded the determined or probable time required for complete recovery of conductivity in the normal or control series. The heavy dots are for series in which the a-R intervals were so short that the junctional tissue did, in all probability, play a part in producing the variations in the A-V intervals. The figure shows that the effect of the blocked impulses was consistently greater in those cases where the compression of the muscle was great, i.e., where the a-R intervals were long. If this difference is not merely accidental owing to the scantiness of the data, it may be attributed to the probability that where the a-R intervals are short, variations in the A-V intervals will occur, not only in the compressed muscle but also in the junctional tissue beyond the clamp. Since the blocked impulse does not reach the junctional tissue, its influence upon the total A-V will be less than in cases where the a-R interval is long, and where therefore the variations in the A-V intervals are due almost wholly to changes in the conductivity of the compressed muscle.

Relation between the length of the a^1-a^2 interval and the effect of blocked impulses. It was assumed at the outset in describing the effect of blocked impulses, that the recovery curves R^1 and R are of the same form (fig. 6 *A*).

The truth of this assumption might be tested with sufficient data, for, if R^1 does not have the same form as R, we would expect that the average effect of the blocked impulses would be greater or less when the a^1-a^2 interval is long than when it is short. By the a^1-a^2 interval is meant the interval which elapses between the blocked and the next transmitted impulse. Figure 7 *D* shows the result of plotting averages of all points for which the a^1-a^2 intervals were 85 per cent or less of the a-R interval (heavy dots); and of all points for which they were 95 per cent or over (circles). These results point to a somewhat slower recovery of conductivity following the blocked than following the transmitted impulse; but again, owing to the scantiness of the data, this conclusion can be offered but tentatively. A not improbable explanation of the apparent slower recovery of conductivity following the blocked impulse is a "fatigue effect" consequent upon the arrival at the compressed muscle of the transmitted impulse and the shortly succeeding blocked impulse. In this connection see the section upon "fatigue effects" given above.

While it is hoped that further work along these lines will make it possible to offer a satisfactory explanation of the phenomena of partial heart-block, such an explanation cannot yet be advanced. Erlanger (11) states that he found it impossible to explain heart-block on the single basis of a refractory period in the injured tissue. With this statement the writer is in full agreement. Nor does it seem possible to explain partial heart-block in terms of the number of fibers which have recovered from an absolute refractory period, a view toward which Lewis (18) appears to be working. Whatever may be the relation between fiber conduction and the relative refractory period in the dog's auricle when under the influence of the vagus (19), the work of Forbes, Ray and Griffith (20), which shows that in nerve at least the impulse is conducted more slowly during the relative refractory period, leaves the possibility that a similar relation may hold for cardiac muscle. Garrey (21) suggested that an explanation of partial heart-block must take account of various factors, among them 1, a refractory period at the site of the block, and 2, fatigue effects. The results herein reported demonstrate the presence of a relative refractory period in conductivity (possibly depending upon excitability changes) and of cumulative "fatigue effects." The writer therefore believes that the two factors mentioned above are important for the explanation of partial heart-block. The other factors mentioned by Garrey, as he clearly recognized, apply under more or less special circumstances. The further fact, which has been demonstrated by our results, that a blocked impulse may exert a profound influence upon conductivity must also not be overlooked if an explanation of partial heart-block is attempted.

I gratefully acknowledge my debt to Dr. W. E. Garrey for encouragement and constructive criticism; to Dr. A. S. Gilson for helpful suggestions; and to Miss Roberta Hafkesbring for aid in obtaining the tracings.

SUMMARY

The experimental work reported above gives results from which the following conclusions may be drawn:

1. In the quiescent, excised, atropinized heart of the turtle, in which the muscle in the auriculo-ventricular groove has been compressed, it is found that after the transmission of an impulse through the compressed muscle there follows a period during which a second impulse will not be transmitted, which is followed in turn by a period during which an impulse will pass more slowly than in the resting muscle. If the second impulse falls early in this period it is transmitted slowly; if later, more rapidly. The form of curve of recovery of *conductivity* thus determined resembles the recovery curve of *excitability* of cardiac muscle found by Trendelenburg, and of *excitability* of nerve determined by Adrian; but we do not consider the interval during which a second impulse will not pass as coterminous with an absolute refractory period.

2. Many curves show cumulative effects of activity attributed to fatigue.

3. An impulse which reaches the compressed muscle during that period when, because of the previous passage of an impulse, a second impulse will not be tranmitted to the ventricle, has, nevertheless, a definite effect in prolonging the duration of a subsequent A-V interval. The earlier the blocked impulse falls, the less is its effect; when later, the effect is greater.

4. Evidence is presented to show that under certain conditions the *variations* in conduction time may be attributed almost entirely to changes in the condition of the compressed muscle.

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CONDUCTIVITY IN COMPRESSED CARDIAC MUSCLE

II. A Supernormal Phase in Conductivity in the Compressed Auricular Muscle of the Turtle Heart

RICHARD ASHMAN

From the Physiological Laboratory of the Tulane University of Louisiana, School of Medicine

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This paper presents evidence for the presence of a supernormal phase in conductivity in compressed auricular muscle of the turtle heart. Phenomena depending upon the existence of a supernormal phase in the excitability, conductivity or contractility of tissues have frequently attracted the attention of physiologists since the pioneer work of Bowditch upon Treppe in the heart. That a Treppe, indicative of a supernormal phase in contractility, may possibly occur in the comparatively normal human ventricle is shown by the case reported by Vinnis (1), who described a Treppe in the ventricular systoles following the compensatory pause after a premature auricular systole. Gaskell (2, p. 106) called attention to a Treppe in the development of the rhythmical power of the excised ventricle of the frog. For nerve, Adrian and Lucas (3) demonstrated that under certain conditions the excitability, instead of returning to normal rapidly at first and then more and more slowly, rose rapidly to or beyond the resting excitability, then more slowly to a maximum from which it gradually declined to the excitability characteristic of the resting nerve. Thus, for a short time following the passage of an impulse a supernormal phase was present, i.e., the excitability of the nerve was greater than the resting excitability. In explaining the phenomenon of summation of impulses (stimuli) in nerve, these investigators proved than an impulse, set up during the supernormal phase, would pass a region of decrement which blocked the normal impulse. From this Adrian and Lucas argued that the impulse was of greater intensity during the supernormal phase, and they took this augmented intensity as a measure of the conductivity. Although unable to show that the recovery of conductivity ran precisely parallel with the return of excitability, Lucas evidently believed such to be the case (4, p. 80).

Adrian, writing the last chapter in Lucas' monograph, "The Conduction of the Nervous Impulse," applied their findings mentioned above in an attempt to explain summation in the central nervous system. Engelmann (5) mentions a Treppe in conductivity in the frog heart, i.e., under

certain conditions the first impulse from auricle to ventricle may pass slowly, the next more rapidly, etc., until a constant A-V interval is reached.

Adrian (6) studied the conditions under which the supernormal phase appears and reported that its development is dependent upon the hydrogen ion concentration of the solution with which the tissue is bathed. The nerve will develop a supernormal phase if placed, for a sufficient time, in a solution of pH 7 or less (acid side of neutrality), while the ventricular muscle of the frog's heart may show evidence of a supernormal phase even if the perfusate is at a somewhat higher pH, i.e., more alkaline. Adrian further reported that the excitability of the nerve during the supernormal phase did not, as a matter of fact, exceed the normal excitability of the same nerve in an alkaline solution; thus the resting excitability in an acid medium is depressed.

In the experiments reported below a supernormal phase in conductivity was obtained. A Treppe in conductivity, which is dependent upon the presence of a supernormal phase, and which manifests itself as a progressive decrease in the A-V intervals after a rest, also frequently made its appearance. The method employed was in all respects similar to that described in the previous paper (I) of this series. It was noted that the supernormal phase was likely to appear after the heart had been experimented with for some time; or, when the weather was warm (35 to 37°C.) the first series of contractions obtained usually showed it. Some of the hearts however developed no supernormal phase in conductivity even after two or more hours of activity while removed from the animal and not perfused. In all cases when the supernormal phase in conductivity was present, the ventricle showed a Treppe in contractility. In some cases the auricular contractions showed a Treppe, in other cases they did not. On the other hand, the ventricular contractions often showed a marked Treppe while there was no supernormal phase in conductivity in the compressed auricular tissue. About 35 measured series from 14 hearts (of about 40 hearts experimented with) show unmistakable supernormal phases in conductivity. The phenomenon of summation of impulses, to be described, was recorded about fifteen times (4 hearts), and observed on several other occasions. A Treppe in conductivity was much more frequently obtained. All curves showing a supernormal phase, with one doubtful exception, were obtained either in fatigued hearts or when the room temperature was high.

Results. A few of the curves which the writer believes demonstrate the presence in the compressed muscle of a supernormal phase in conductivity are presented in figures 1, 2 and 3. Figure 1 is constructed from measurements of a series of contractions from an excised, atropinized turtle heart which had been experimented with for an hour or two previously. The abscissae represent time intervals between auricular sys-

toles, and the ordinates the consequent A-V intervals. This curve shows clearly that an impulse arriving at the compressed region less than 2.5 seconds after the passage of a previous impulse is transmitted slowly; that an impulse arriving during the next second or after the end of about 10 seconds is transmitted more rapidly; and that an impulse reaching the block during the interval from about 3.5 to 10.0 seconds after the passage of a previous impulse is transmitted still more rapidly; while an impulse arriving at the end of 6 or 7 seconds is transmitted most rapidly. The numbers of the points indicate the sequence in which they occur in the series.

Figure 2 shows three other curves similarly obtained and plotted. Curve *A* is double, since following point *9* in the series a sudden change in conductivity occurred. Each part of the series, however, shows clearly a

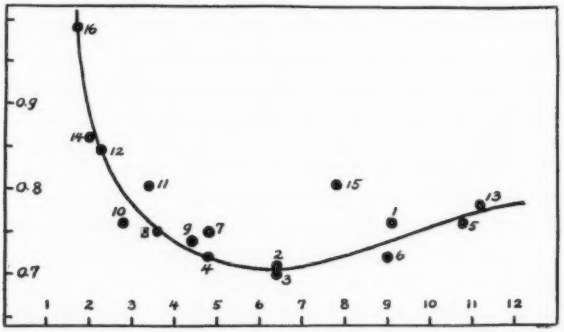

Fig. 1. Curve showing a supernormal phase in conductivity plotted from measurements of series 5 of January 10, 1925. Abscissae represent intervals between auricular contractions in seconds; ordinates, the A-V intervals in seconds. The numbers of the points indicate the order in which the contractions occurred in the series. Temperature, 23°C.

supernormal phase in conductivity. For further explanation see the legend. Figure 3 was obtained from a ventricular strip and is discussed below.

Early in our work we felt justified in predicting that if, during the time the supernormal phase occurred, the heart should be brought to a stage of block just complete for impulses arriving at a low frequency, i.e., at intervals of 15, 20 seconds or more, an impulse sent in during the supernormal phase following the blocked impulse might succeed in passing. The realization of this prediction is shown in figure 4, *A* and *B*. The tracing *A* shows that an impulse reaching the compressed region after a relatively long rest fails of transmission (auricular contraction 1). The next impulse, that of auricular contraction 2, arriving about 3.6 seconds later, likewise fails. The third impulse, 3.4 seconds after the second, passes the block and causes a

ventricular response. After another long rest, impulse 4 was sent in and failed to pass; but impulse 5, 4.2 seconds later, gets through to the ventricle. The next group of responses in this tracing is similar to the first. Figure 4 *B* is another example of the same phenomenon obtained from a ventricular strip. The block, in this case, was produced by cutting at the middle of the strip so as to leave a narrow bridge of muscle and by treating

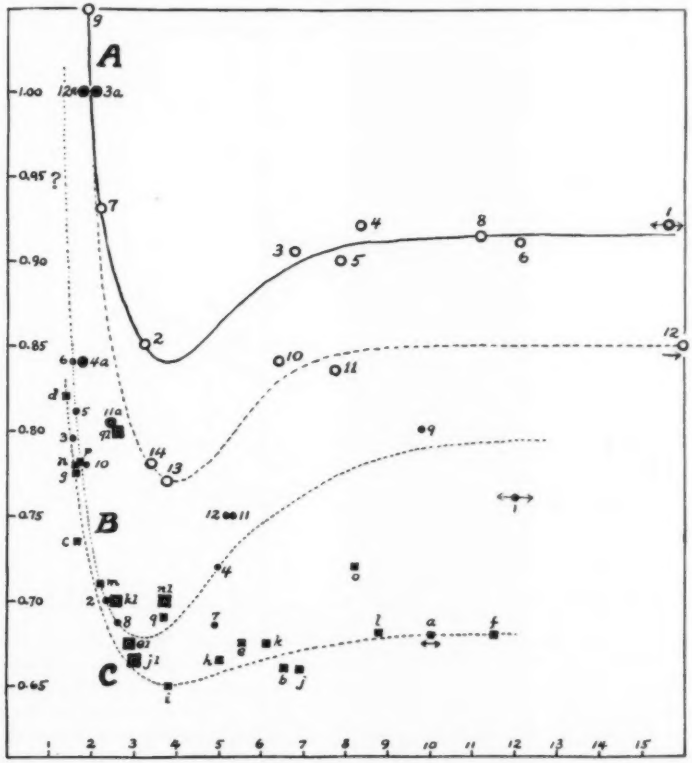

Fig. 2. Examples of the supernormal phase in conductivity. Abscissae, interauricular intervals; ordinates, A-V intervals in seconds. *A*, Series 7 of June 3, 1924. Following point *9* a sudden shift in the durations of the A-V intervals occurred, causing a double curve, each part of which shows the supernormal phase. *B*, Series 12, May 26, 1924. Points as small solid circles. The large solid circles represent 2:1 blocks and follow the points of corresponding number (see first paper of this series for explanation of blocks). *C*, Series 4, June 3, 1924. Points as small squares, lettered in sequence. Large squares represent 2:1 blocks. The curve is from the same heart as *A* and represents an earlier stage in the development of the supernormal phase. Temperatures unrecorded.

the bridge with an alcohol-Ringer mixture (5 to 10 per cent alcohol). This tracing shows the further fact that if one impulse succeeds in passing, all following impulses, if the rest period is not too long and barring fatigue, will also pass. Figure 1, besides serving as an example of the supernormal phase in conductivity, proves that such a condition was actually present in the muscle at the time figure 4 *A* was recorded, since the series from

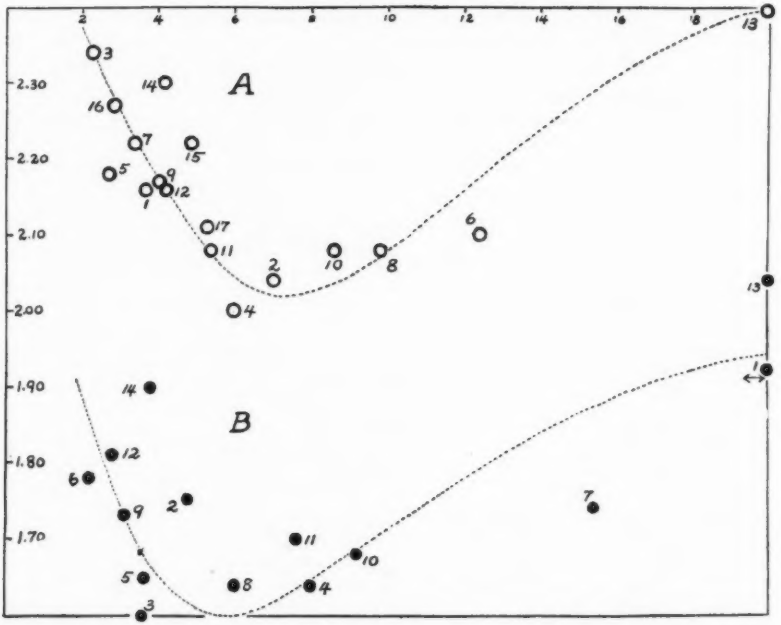

Fig. 3. Curves showing a supernormal phase in conductivity from a ventricular strip. January 21, 1925. Abscissae represent the time intervals between the contractions of the end of the ventricle stimulated; ordinates the interval between the beginning of the contraction of the end of the strip stimulated and of the part beyond the clamp. The points are numbered in the sequence in which they occurred. Note the Treppe in conductivity (*A* 13, 14, 15, 16, 17); and the cumulative fatigue effects (*B* 8, 9, 10, 11, 12). Temperature, 24°C.

which figure 1 is derived was obtained in the same heart a few minutes after figure 4 *A*. So marked a supernormal phase could hardly have developed in those few minutes, particularly at the temperature, 25.5°C. Figure 3, *A* and *B*, taken from the ventricular strip, likewise serve as controls for figure 4*B*, proving that a supernormal phase in conductivity was actually present when the phenomenon of summation of impulses, above described, was obtained.

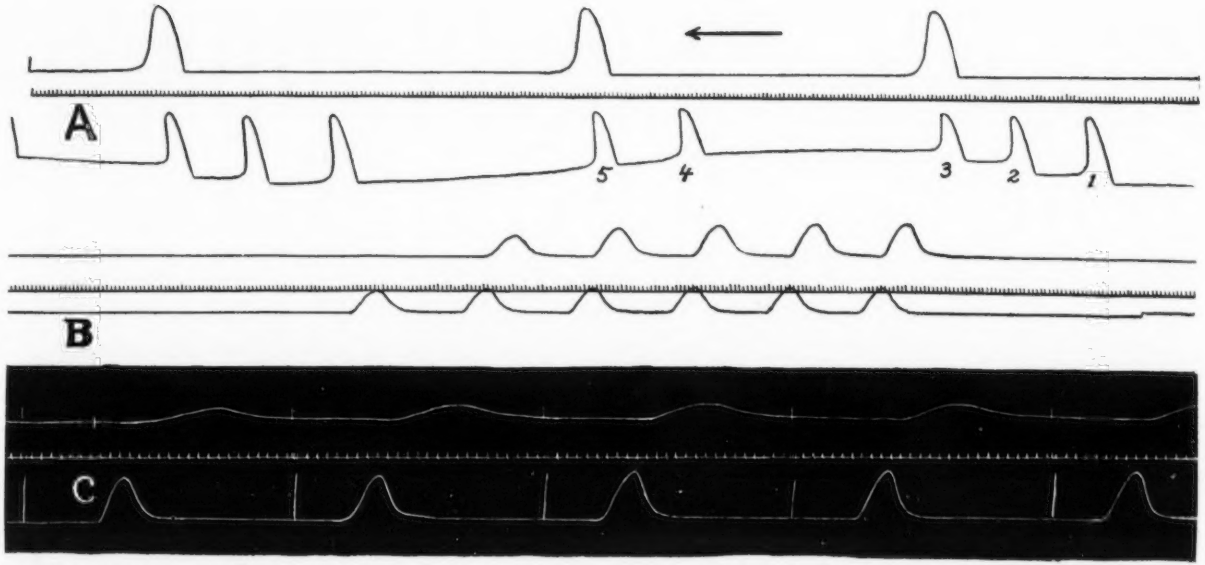

Fig. 4. *A*, Summation of impulses, January 10, 1925. To be read from right to left. *B*, Summation of impulses in ventricular strip. *C*, The first three A-V intervals in this tracing show a Treppe in conductivity. The last two show the onset of fatigue. The initial rest period was about 70 seconds, while the auricular contractions (below) are spaced at 5 second intervals. The durations of the A-V intervals in the order of their occurrence are 1.15, 1.00, 0.95, 0.96, 0.97 seconds. June 6, 1925. Room temperature, 29°C. Time in fifths. (In *C*, necessary retracing, done in offices of the publisher, obscures the true time relations.)

In figure 4 *C* is illustrated a Treppe in conductivity, a phenomenon of which a considerable number of examples have been obtained. It is really the same phenomenon as the summation of impulses, excepting that the compression was not sufficiently great to prevent the passage of the first impulse after a long pause. The first A-V interval, after a relatively long rest, is long, the next A-V is shorter, the next still shorter, i.e., a Treppe in conductivity is shown. Another example of this phenomenon is to be seen in figure 3 *A*, points *14*, *15*, *16* and *17*. The scattering of the points in these curves appears to result from a combination of Treppe and of fatigue effects.

In addition to the phenomena already mentioned, some of the series showing a supernormal phase also show unmistakable cumulative fatigue effects similar to those described in the first paper of this series. See also figure 3 of this paper. On June 2, 1925, an interesting combination of Treppes, of summations of impulses, and of fatigue effects was obtained. For about an hour the degree of compression applied by the clamp remained unchanged, and during this period it was found that a single impulse reaching the compressed muscle during an interval of from 15 to about 60 seconds following the passage of a previous impulse was blocked, while a later or earlier impulse, if not too early, was transmitted. Analysis of the tracings showed clearly that the blocking of the impulse was due to fatigue from which the muscle recovered sufficiently during a minute or more of rest to permit a single impulse to pass. But if a properly timed series of impulses was sent in during the interval when fatigue prevented the passage of the single impulse, the second, third or fourth impulse of the series invariably passed (summation of impulses), while if the impulses of the series continued to arrive at the same rate a partial block developed (fatigue). An extensive analysis of these relationships is planned for a forthcoming paper.

Discussion. Our results have clearly brought out the fact that a supernormal phase in conductivity may exist in the compressed cardiac muscle, although it must be granted that the improvement in conductivity may depend upon a supernormal phase in the excitability of the muscle elements concerned in the conduction of the impulse. Several phenomena depending upon the presence of the supernormal phase deserve separate mention.

1. A Treppe in conductivity. In a considerable number of series a Treppe in conductivity is to be found. The similarity of this condition to a Treppe in contraction is obvious. In one of Skramlik's papers (7) in the last figure, the last two contractions show a Treppe in conductivity between bulbus and ventricle. Engelmann mentions this phenomenon in a review published in the Deutsche Klinik (5).

2. Summation of impulses. This phenomenon is, like the Treppe,

dependent upon a supernormal phase in conductivity, and is, in reality, a form of the staircase. With a degree of block which just prevents the passage of an impulse set up in the resting heart, a pair or a series of impulses will lead to a ventricular response if the supernormal phase is present in the compressed region. Drury (8) and Drury and Andrus (9) have recently given reasons why the impulse in compressed auricular muscle or in muscle bathed in solutions of increased hydrogen ion concentration may be regarded as undergoing a decrement in its conduction. If it be assumed therefore that conduction with a decrement in compressed cardiac muscle is a fact, the explanation of the summation of impulses would be as follows. The impulse reaching the block after a long rest, although failing to pass through, penetrates to a certain distance. The next impulse, arriving during the supernormal phase left by the first, is conducted more easily and penetrates the compressed muscle still farther. Thus, if the block is not too profound, one of the impulses in the series will pass and a ventricular response will result. Barring fatigue, all subsequent impulses will be transmitted if the rest period is neither too long nor too short. This explanation assumes the fact of conduction with a decrement. In this connection it should be noted that Davis, Forbes, Brunswick and Hopkins (10) have very recently reported that in narcotized nerve there is no conduction with a decrement. They state that such reduction in magnitude as the impulse undergoes in the anesthetized region is sudden and occurs when the impulse enters that region. Unless the impulse is then extinguished it progresses, without further diminution, through the narcotized region and regains its normal intensity upon reëntering normal nerve. In view of these results the above explanation of summation of impulses at a block in cardiac muscle must be recognized as purely tentative.

That the success of the impulse in passing the compressed region does not depend upon the magnitude of the auricular impulse reaching that region seems clear. This is indicated by figure 3 *A* which shows no Treppe in contractility in the auricle; but it is more clearly shown in a recent paper by Lewis and Master (11) who report a supernormal phase in the human heart. Since the auricular rhythm was regular in the first case reported by these investigators the success of the impulse in passing a block was not associated with variations in the intensity of the arriving impulses.

Attention should be directed to the fact that the summation of impulses as it is observed in blocked cardiac muscle is different in one essential feature from summation in nerve as described by Adrian and Lucas (3). In the experiments of those investigators the impulse set up during the supernormal phase in excitability in nerve succeeded in passing a narcotized region which extinguished the normal impulse, not because of changes in conductivity occurring in the region of block, but as a result of changes in the magnitude of the impulse in the unanesthetized nerve. In case

of summation in the compressed cardiac muscle on the contrary, the changes take place in the compressed muscle itself. Forbes' (12) theory of summation in the central nervous system, a development of the theory proposed by Lucas (4), appears to invoke a mechanism analogous to that obtaining in the heart.

3. Fatigue effects and the supernormal phase. The occurrence of "fatigue effects," described in the first paper of this series, i.e., effects which result in a progressive increase in the conduction time when impulses arrived at the compressed region in rapid sequence, even in tissue showing a supernormal phase in conductivity, may indicate that the relative refractory period, of which the supernormal phase may be a manifestation, and fatigue have fundamentally different causes.

Discussion of the results of others. Skramlik (7), in a turtle heart which had been brought to a standstill by the first Stannius ligature, produced, by warming the auriculoventricular junction, a degree of block which prevented the passage of impulses from ventricle to auricles, while impulses could readily pass in the usual direction. If conditions were right he found that after several impulses had been sent from auricles to ventricle, impulses would then pass in the reverse direction providing he did not wait too long before stimulating the ventricle. Once an impulse had passed in the backward direction, any number would pass if the rest interval was not too long. He found the same relation to hold between the bulbus and the ventricle. This phenomenon Skramlik named "die Bahnung der Erregung." It receives a ready interpretation on the basis of a supernormal phase in conductivity, i.e., the auricular impulses which passed left the whole pathway between the two chambers in a supernormal state, and thus the ventricular impulse, previously blocked, could pass. Why repeated ventricular stimulation did not lead ultimately to transmission from ventricle to auricles is not clear; the explanation, no doubt, depends upon an adequate elucidation of irreciprocal conduction. It is, however, difficult to understand why Skramlik failed (if in fact he did fail) to obtain a degree of block which would permit the last of a properly time series of auricular impulses to pass.

Lewis and Master (11) report two clinical cases of heart-block which they believe demonstrate the presence in the human heart of a supernormal phase. Their first case was, in a sense, a functionally complete block. Auricular impulses, initiated in all phases of the cardiac cycle, were blocked, excepting those sent out from the sinus node during an interval extending from 0.425 to about 0.700 second after the beginning of the R wave of the electrocardiogram. Impulses falling early during this interval were transmitted with a P-R interval of about 0.12 second, while those falling at the end of the interval gave a P-R of about 0.168. Thus the later the impulses arrived, during the short interval when con-

duction was possible, the longer the conduction time. In explanation Lewis and Master say (p. 378),

> We suppose, therefore, that this clinical patient exhibits the phenomenon displayed by nerve and muscle bathed by fluids which are relatively acid; we suppose further that the line of recovery as a whole is depressed. During the later phases of diastole the state of recovery is such that the natural impulses are inadequate; only during a short and early period of diastole, where there is an overswing of the recovery curve, and where it consequently crosses the threshold line, are they adequate.

They further conclude that the junctional tissue, between the auricles and auriculo-ventricular node, is at fault. That their explanation is correct is rendered more probable from the experimental results obtained by us which prove that a supernormal phase in conductivity may exist in the heart, and that where there is a sufficient depression of conductivity only those impulses falling near or upon the crest of the supernormal recovery curve can be transmitted. In one important respect this supernormal phase as described by Lewis and Master, is different from the supernormal phase in conductivity as herein described, i.e., the phase is presumably produced, not by an impulse which is transmitted through the tissue in question, but by an impulse arising in the A-V node, an impulse blocked off, by irreciprocal conduction, from the auricle. It should therefore be possible in occasional cases to imitate this condition experimentally, i.e., by obtaining a block against impulses in either direction, but in which an impulse reaching the block from one side will facilitate the passage of an impulse arriving from the other side. The evidence of a supernormal phase in the second case described by Lewis and Master is not convincing. While the time relations are such that its possible presence cannot be disproved, the condition illustrated seems susceptible of ready explanation on other and more usual grounds.

The writer wishes to acknowledge his indebtedness to Prof. W. E. Garrey for invaluable suggestions and criticisms.

SUMMARY

1. A supernormal phase in conductivity is described. The form of the curve showing the supernormal phase is strikingly like those for the excitability of nerve (Adrian and Lucas) and for the excitability and contractility of heart muscle (Adrian).

2. In consequence of the presence of a supernormal phase, an impulse arriving at a compressed region in the auricular muscle at a certain time after a previous impulse has passed will be transmitted more rapidly than one arriving earlier or later.

3. By proper adjustment of the clamp a degree of compression of the muscle may be established such that only those impulses falling on or near the crest of the supernormal phase will be transmitted through the compressed region.

4. A Treppe in conductivity, characterized by a progressive decrease in the A-V interval for a series of impulses following a long rest period, is described; and, under the conditions mentioned in 3 above, a phenomenon of summation of impulses also makes its appearance.

5. These phenomena are believed to be independent of changes in the condition of the uncompressed muscle, i.e., they depend upon the state of the compressed auricular muscle.

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NORMAL VARIATIONS OF PERCENTAGE WEIGHTS OF BODY ORGANS OF THE ALBINO RAT WITH CHANGING BODY WEIGHT

A. T. CAMERON

From the Department of Biochemistry, Faculty of Medicine, University of Manitoba, Winnipeg, Canada

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Dr. H. H. Donaldson (1923) has recently drawn attention to the normal variations of the body organs of the rat, expressed in percentages of body weight, with changing body weight, and has pointed out the necessity of considering such changes in nutritional and pharmacological studies in which the rat is employed as experimental animal.

In work which endeavours to utilise the degree of production of hypertrophy of body organs, such as is produced by thyroid feeding, as a measure of activity of such thyroid, it is obviously necessary to apply a correction for normal variations, since one of the marked effects produced by thyroid feeding is a lessened growth-rate, so that at the end of an experiment there may be a marked difference between the weights of controls and of thyroid-fed animals.

In his paper Donaldson has given curves for the percentage variations of different organs referred to body weight, taking for each organ the value at birth as unity. Such curves are not very suitable for use in applying a correction in studies such as I have been making. I have accordingly constructed curves of the actual percentage variations with body weight from the actual weight figures given by Donaldson in *The Rat* (1924). These curves have been tested with my own results from normal animals, used as controls and dissected and weighed in experiments during the last four and one-half years.[1] There are certain marked discrepancies which it seems desirable to place on record, and which necessitate the utilization of curves constructed from my own results for certain of the body organs whose changes in experimental animals I have used as criteria.

One cause of disagreement between my own figures, and those given in *The Rat* is probably diet. My own animals were usually separated and fed a bread and milk diet only, for several weeks before dissection. In those of lower body weight this restricted diet was only given for a week

[1] Results obtained prior to December, 1920, have not been included.

before dissection, while the heaviest rats had been fed this diet for several months.

The organs were not weighed so accurately as those for which Donaldson publishes results. Much of the dissection was carried out in the winter months, in which, in this climate, there is practically no moisture in the atmosphere, so that exposed organs show visible "drying out" within a few minutes. Speed of dissection and transference to closed glass vessels for weighing has therefore been essential, and nothing was to be gained by extreme accuracy in weighing, since, through such speed, some degree of dissection error was unavoidable. Livers were weighed to 0.1 gram, kidneys, hearts and testes to 0.01 gram, spleens, adrenals, lymph glands and muscle to 0.001 gram, and thyroid to 0.0001 gram (and I doubt if the last figure for thyroids has much significance). The body weights are correct to within 0.5 gram. Stomach and intestinal contents were always neglected, and since these varied a slight error arises from this variation.

Livers were dissected by clamping the vena cava just below the diaphragm, and pulling the liver away gently from the other abdominal contents. In this way livers were obtained filled with blood, which presumably accounts for the average higher percentage value than that obtained from Donaldson's figures. Hearts were dissected out at the junction of the great vessels, cut open, and roughly dried with filter paper.

The muscle selected for examination was the right tibialis anterior. The lymph glands dissected were those of the anterior triangles of the neck.

Results have been grouped for body-weight variations increasing by about 10-gram intervals, and the averages referred to the average body weight of each group. These grouped results (extremes in parentheses: averages italicized) are given in table 1 (males) and table 2 (females), excluding those for lymph glands. Corresponding values calculated from Donaldson's figures in *The Rat*, over a similar range of body-weights, are given in table 3.

When curves, drawn from the figures in tables 1 and 2, showing variation of percentage organ weight with changing body weight, are compared with similar curves constructed from Donaldson's figures (table 3), those for kidneys and hearts of both sexes and for adrenals of male rats approximate fairly closely. On the other hand muscle (right anterior tibialis) and thyroid of both sexes and adrenals of female rats appear to be proportional to body weight over a considerable range of the latter, indicating that no correction need be applied over this range to such tissues in animals living under the conditions in these experiments.

No similar figures are available for single muscles of closely confined rats. Such a result for muscle is not in agreement with Jackson's results for total musculature. The figures for thyroid are uniformly lower than those given by Donaldson.

Figures for testes are extremely variable; while they tend to a maximum

TABLE 1
Male rats

TABLE 1

Male rats

NUMBER OF RATS	BODY WEIGHT	PERCENTAGE WEIGHTS							
		Liver	Kidneys	Heart	Spleen	Adrenals	Testes	Muscle	Thyroid
	grams	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
2	(47–48)	(6.2–7.1)	(1.12–1.45)	(0.48–0.55)	(0.29–0.31)	(0.028–0.029)	(0.96–1.00)	(0.194–0.204)	(0.0111–0.0131)
	47.5	*6.7*	*1.29*	*0.52*	*0.30*	*0.0285*	*0.98*	*0.199*	*0.0121*
6	(61–68)	(6.2–7.1)	(0.95–1.10)	(0.46–0.54)	(0.27–0.39)	(0.019–0.027)	(0.48–1.33)	(0.177–0.194)	(0.0074–0.0118)
	65	*6.5*	*1.02*	*0.50*	*0.34*	*0.025*	*0.97*	*0.182*	*0.0086*
3	(75–82.5)	(5.9–6.4)	(0.96–1.08)	(0.47–0.49)	(0.31–0.34)	(0.013–0.024)	(0.56–1.48)	(0.160–0.205)	(0.0091–0.0114)
	78	*6.2*	*1.00*	*0.48*	*0.32*	*0.018*	*0.97*	*0.186*	*0.0099*
4	(91–98)	(5.8–6.2)	(0.95–1.00)	(0.41–0.49)	(0.25–0.34)	(0.018–0.020)	(0.98–1.82)	(0.188–0.208)	(0.0089–0.0124)
	95	*6.0*	*0.98*	*0.45*	*0.29*	*0.019*	*1.60*	*0.200*	*0.0108*
4	(100.5–109)	(5.9–6.5)	(0.93–1.05)	(0.40–0.54)	(0.26–0.43)	(0.019–0.022)	(0.66–1.83)	(0.184–0.206)	(0.0073–0.0139)
	105	*6.3*	*1.00*	*0.47*	*0.31*	*0.020*	*1.22*	*0.194*	*0.0103*
7	(111.5–119.5)	(5.4–7.4)	(0.93–1.08)	(0.39–0.48)	(0.26–0.38)	(0.013–0.021)	(0.72–1.93)	(0.187–0.223)	(0.0073–0.0172)
	115	*6.5*	*1.01*	*0.45*	*0.33*	*0.018*	*1.41*	*0.197*	*0.0121*
4	(122–128)	(6.0–7.1)	(0.89–1.02)	(0.42–0.49)	(0.21–0.35)	(0.016–0.020)	(1.11–1.73)	(0.182–0.202)	(0.0092–0.0124)
	126	*6.5*	*0.95*	*0.46*	*0.29*	*0.018*	*1.44*	*0.195*	*0.0105*
7	(130–135.5)	(5.9–6.5)	(0.84–1.06)	(0.38–0.44)	(0.24–0.38)	(0.019–0.029)	(0.94–1.62)	(0.176–0.204)	(0.0088–0.0185)
	133	*6.2*	*0.94*	*0.42*	*0.29*	*0.023*	*1.30*	*0.189*	*0.0125*
3	(139–144)	(5.6–6.6)	(0.87–1.01)	(0.38–0.40)	(0.26–0.37)	(0.014–0.029)	(1.10–1.43)	(0.182–0.195)	(0.0080–0.0126)
	141	*6.2*	*0.92*	*0.39*	*0.30*	*0.021*	*1.24*	*0.189*	*0.0102*
8	(150–162)	(4.9–6.5)	(0.81–0.97)	(0.38–0.45)	(0.22–0.29)	(0.014–0.020)	(1.14–1.60)	(0.159–0.199)	(0.0062–0.0133)
	158	*5.8*	*0.91*	*0.40*	*0.26*	*0.017*	*1.37*	*0.183*	*0.0104*
6	(166.5–176.5)	(5.1–7.0)	(0.83–1.09)	(0.31–0.41)	(0.25–0.29)	(0.014–0.021	(1.21–2.11)	(0.167–0.190)	(0.0089–0.0136)
	170	*6.1*	*0.93*	*0.37*	*0.27*	*0.017*	*1.50*	*0.176*	*0.0111*
1	*192*	*7.7*	*0.87*	*0.56*	*0.23*	*0.017*	*1.14*	*0.193*	*0.0135*
4	(255–279.5)	(4.3–5.3)	(0.84–0.94)	(0.33–0.37)	(0.15–0.19)	(0.010–0.013)	(0.76)	(0.168–0.207)	(0.0050–0.0112)
	271	*4.9*	*0.88*	*0.35*	*0.17*	*0.011*	*0.76*	*0.189*	*0.0076*
4	(288–307)	(4.7–5.8)	(0.77–0.97)	(0.29–0.36)	(0.14–0.17)	(0.008–0.013)	(0.80–0.83)	(0.176–0.200)	(0.0066–0.0108)
	295	*5.1*	*0.84*	*0.32*	*0.15*	*0.011*	*0.81*	*0.190*	*0.0088*

TABLE 2

Female rats

NUMBER OF RATS	BODY WEIGHT	PERCENTAGE WEIGHT						
		Liver	Kidneys	Heart	Spleen	Adrenals	Muscle	Thyroid
	grams	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	*31*	*7.3*	*1.32*	*0.61*	*0.31*	*0.032*	*0.136*	*0.0110*
1	*44*	*6.8*	*1.18*	*0.59*	*0.27*	*0.027*	*0.207*	*0.0118*
4	(52–58.5)	(6.1–7.3)	(1.06–1.20)	(0.48–0.56)	(0.25–0.38)	(0.027–0.031)	(0.172–0.210)	(0.0100–0.0140)
	54	*6.5*	*1.14*	*0.52*	*0.33*	*0.029*	*0.190*	*0.0125*
4	(62–70)	(5.7–7.0)	(1.00–1.14)	(0.50–0.55)	(0.23–0.34)	(0.017–0.024)	(0.155–0.290)	(0.0070–0.0156)
	66.5	*6.3*	*1.06*	*0.52*	*0.29*	*0.021*	*0.212*	*0.0105*
5	(76–79)	(6.1–7.1)	(1.03–1.25)	(0.42–0.54)	(0.28–0.39)	(0.019–0.030)	(0.195–0.228)	(0.0113–0.0152)
	77.5	*6.5*	*1.10*	*0.49*	*0.34*	*0.025*	*0.207*	*0.0130*
8	(80–5–84.5)	(5.7–8.0)	(0.95–1.12)	(0.45–0.57)	(0.24–0.37)	(0.026–0.033)	(0.168–0.212)	(0.0098–0.0162)
	83	*6.5*	*1.01*	*0.49*	*0.32*	*0.028*	*0.196*	*0.0125*
8	(87–96)	(4.5–7.6)	(0.80–1.10)	(0.40–0.60)	(0.24–0.35)	(0.015–0.032)	(0.184–0.230)	(0.0076–0.0141)
	92	*6.0*	*0.99*	*0.46*	*0.28*	*0.024*	*0.202*	*0.0112*
8	(96.5–105)	(5.7–7.5)	(0.91–1.10)	(0.44–0.49)	(0.28–0.46)	(0.024–0.034)	(0.173–0.200)	(0.0083–0.0157)
	101.5	*6.7*	*0.98*	*0.46*	*0.35*	*0.029*	*0.188*	*0.0112*
9	(107–115)	(4.6–7.8)	(0.85–1.11)	(0.38–0.54)	(0.25–0.37)	(0.022–0.039)	(0.167–0.211)	(0.0096–0.0139)
	110	*6.2*	*0.97*	*0.47*	*0.31*	*0.027*	*0.193*	*0.0118*
9	(120–131.5)	(4.6–8.5)	(0.72–1.13)	(0.37–0.48)	(0.23–0.34)	(0.022–0.037)	(0.178–0.202)	(0.0089–0.0142)
	125.5	*6.0*	*0.98*	*0.43*	*0.28*	*0.030*	*0.193*	*0.0114*
2	(139–145)	(5.9–6.9)	(0.95–0.98)	(0.36–0.44)	(0.24–0.26)	(0.024–0.028)	(0.175–0.205)	(0.0079–0.0171)
	142	*6.4*	*0.97*	*0.40*	*0.25*	*0.026*	*0.190*	*0.0125*
3	(175.5–179.5)	(4.9–5.7)	(0.84–0.99)	(0.38–0.47)	(0.17–0.20)	(0.027–0.030)	(0.189–0.212)	(0.0105–0.0136)
	177	*5.3*	*0.92*	*0.42*	*0.19*	*0.028*	*0.203*	*0.0118*
2	(202–224)	(5.2–5.5)	(0.90–1.02)	(0.35–0.36)	(0.17–0.17)	(0.023–0.025)	(0.178–0.185)	(0.0069–0.0112)
	213	*5.4*	*0.96*	*0.36*	*0.17*	*0.024*	*0.182*	*0.0090*

at about 100 grams body-weight in agreement with Donaldson's figures the exceptions are very numerous. I have been unable to relate the variations to any factor such as period of year or age.

The smoothed curves for liver and spleen lead to figures somewhat different to Donaldson's and these are included in table 3.

No comparison is available for lymph-glands. Naturally, considering the probable variation in position, considerable individual variations may be expected in such a restricted area as has been selected. But when these lymph-gland percentages are averaged for groups of animals of similar weights and compared with the averages of spleens for the same group, they show the reasonably constant ratio displayed in table 4.

TABLE 3

Percentage organ weights

BODY WEIGHT	FROM DONALDSON'S FIGURES IN "THE RAT"								FROM TABLES 1 AND 2	
	Both sexes					Male adrenals	Female adrenals	Testes	Both sexes	
	Liver	Kidneys	Heart	Spleen	Thyroid				Liver	Spleen
grams	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
30	7.73	1.24	0.624	0.302	0.0244	0.0356	0.0354	0.54		
50	7.16	1.097	0.561	0.297	0.0222	0.0293	0.0321	0.81	6.70	0.32
75	6.52	1.004	0.508	0.287	0.0202	0.0252	0.0301	1.18	6.45	0.315
100	6.02	0.948	0.473	0.280	0.0189	0.0222	0.0288	1.30	6.25	0.31
125	5.68	0.913	0.445	0.275	0.0180	0.0203	0.0279	1.26	6.05	0.29
150	5.41	0.889	0.427	0.272	0.0172	0.0190	0.0273	1.21	5.88	0.27
175	5.19	0.869	0.413	0.269	0.0166	0.0179	0.0266	1.15	5.70	0.25
200	5.01	0.854	0.401	0.267	0.0161	0.0170	0.0265	1.09	5.50	0.225
250	4.75	0.832	0.382	0.264	0.0154	0.0158	0.0258	0.98	5.20	0.18
300	4.54	0.816	0.368	0.261	0.0147	0.0149	0.0256	0.89	4.90	

In order to apply a correction easily from an experimental animal to a control, curves can be constructed of which figure 1 is an example (for kidneys, from Donaldson's figures).

The individual figures on which tables 1, 2 and 4 are based have been deposited with the Wistar Institute.

The normality of the rats used in determining these results (except for variations resulting from the restricted bread and milk diet) is emphasized when the following "pathological" records for the four and one-half years' experiments are considered (excluding effects definitely attributable to treatment).

During the dissection of 412 rats, including 133 controls, 224 fed thyroid preparations, and 55 fed other preparations, the following abnormal results only have been noted:

A "normal" rat, aged 54 days, weight 98 grams, and a young rat of

another litter that had been fed adrenal cortex, showed large cystic left kidneys, with but a trace of kidney tissue remaining. A thyroid-fed rat, dead in tetany, showed both kidneys large and pale; these were examined by Dr. D. Nicholson, who reported subacute diffuse nephritis. A "normal' rat, after prolonged bread and milk diet, fell off in weight and developed

TABLE 4

Comparison of spleen and lymph glands

MALE RATS					FEMALE RATS				
Number of rats	Average body weight	Spleen	Lymph glands	Ratio	Number of rats	Average body weight	Spleen	Lymph glands	Ratio
	grams	*per cent*	*per cent*			*grams*	*per cent*	*per cent*	
2	47.5	(0.29–0.31)	(0.056–0.060)		1	44	*0.27*	*0.098*	0.36
		0.30	*0.058*	0.19	4	54	(0.25–0.38)	(0.081–0.121)	
6	65	(0.27–0.39)	(0.086–0.118)				*0.33*	*0.098*	0.30
		0.37	*0.097*	0.29	3	65	(0.25–0.34)	(0.083–0.095)	
1	75	*0.34*	*0.097*	0.29			*0.30*	*0.090*	0.30
1	97	*0.29*	*0.158*	0.55	8	81.5	(0.24–0.37)	(0.073–0.140)	
5	115	(0.26–0.34)	(0.041–0.102)				*0.315*	*0.103*	0.34
		0.31	*0.064*	0.21	5	93.5	(0.30–0.37)	(0.071–0.150)	
2	128	(0.27–0.29)	(0.056–0.080)				*0.35*	*0.112*	0.32
		0.28	*0.068*	0.24	3	102	(0.30–0.35)	(0.068–0.107)	
3	133.5	(0.24–0.29)	(0.077–0.102)				*0.32*	*0.088*	0.28
		0.26	*0.086*	0.33	3	111	(0.27–0.32)	(0.102–0.145)	
2	142.5	(0.26–0.28)	(0.055–0.096)				*0.29*	*0.127*	0.44
		0.27	*0.075*	0.28	4	124	(0.27–0.31)	(0.093–0.101)	
7	156	(0.22–0.28)	(0.071–0.107)				*0.29*	*0.096*	0.33
		0.26	*0.086*	0.33	2	131	(0.23–0.25)	(0.081–0.093)	
5	171	(0.25–0.29)	(0.065–0.084)				*0.24*	*0.087*	0.36
		0.27	*0.070*	0.26	2	142	(0.24–0.26)	(0.109–0.124)	
1	192	*0.23*	*0.136*	0.59			*0.25*	*0.116*	0.46
4	271	(0.15–0.19)	(0.047–0.062)		3	177	(0.17–0.20)	(0.063–0.068)	
		0.17	*0.054*	0.32			*0.19*	*0.066*	0.35
4	295	(0.14–0.17)	(0.037–0.058)		2	213	(0.17–0.17)	(0.044–0.061)	
		0.15	*0.047*	0.31			*0.17*	*0.052*	0.31
43	Average			0.30	41	Average			0.34

contagious pneumonia (autopsied and reported by Dr. Nicholson). A rat twice fed thyroid during two separate periods of several weeks died subsequently of advanced septic pneumonia. Figures for the abnormal "normal" rats have not been used in the tables given in this paper.

Two normal rats, two thyroid-fed rats, one fed parathyroid, and two fed thyroid and adrenal cortex all developed a pus gland from one of the anterior lymph glands of the neck. No accompanying abnormality was noted.

A litter of eleven rats (of which one died early), born in February, 1922, were all small and weak. One, used as a control, died at age of 54 days, weighing only 30.5 grams. The cause of death was not ascertained. A second control at autopsy weighed only 45.5 grams, and showed consider-

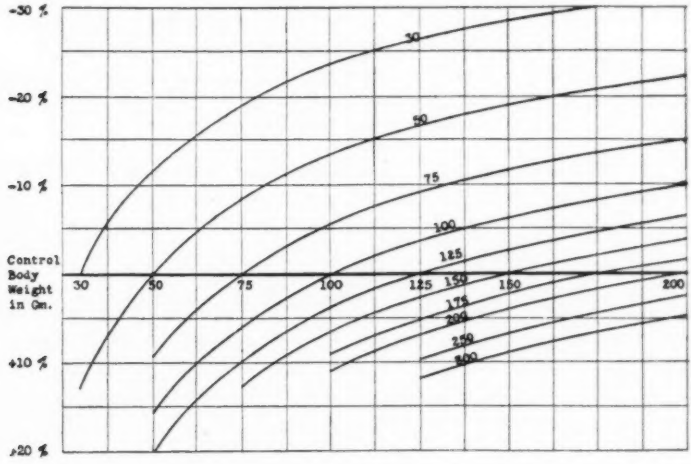

Fig. 1. Correction curves for kidneys constructed from the curve relating percentage weight of kidneys to body weight based on Donaldson's figures. To correct the percentage weight of kidneys of an experimental animal weighing 65 grams to a normal control weighing 75 grams find the vertical distance from 75 on the base line to the (imaginery) curve for 65. This distance is approximately 3.5. Hence subtract 3.5 per cent of the percentage value found for the experimental animal from that value. The correction from an experimental animal weighing 120 grams to a control weighing 105 grams is, similarly determined, 2.4 per cent of, added to the percentage value of the experimental animal.

ably enlarged thyroid, liver, kidneys, and heart. Its condition suggested hyperthyroidism. Unfortunately the thyroid was not examined microscopically. The results with the controls of this litter have been excluded.

I desire to acknowledge my indebtedness to Doctor Nicholson of the Department of Pathology of this University for examining the two abnormal rats mentioned, and to Mr. J. Carmichael for assistance throughout these experiments.

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THE ELECTROGRAM OF THE VENTRICLE

S. DE BOER

From the Pathological Laboratory, Amsterdam

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Before the string galvanometer was used an explanation had already been given for the deflections of the electrogram of the ventricle. Both English investigators, Burdon-Sanderson and Page (1879–1880), were of the opinion that the deflections of the electrogram of the ventricle (they meant the deflections which, following Einthoven, we call R. and T.) are created as the interferential product of the basal and the apical negativity. If they warmed the apex of the ventricle or cooled the base, the height of the T-deflection increased; if however they warmed the base or cooled the apex of the ventricle the T-deflection decreased and at the end was inverted and negative (turned downward). Bayliss and Starling (1892) confirmed these results.

This explanation was again advanced by Boruttau (1913), Mines (1913) and Samojloff in 1913. Samojloff made an incision across the ventricle of the frog's heart so that the basal and apical parts were connected by a narrow muscle bridge. The electrograms of the base and the apex followed then one after the other. He was thus able to separate the components of the ventricular electrogram.

I contributed to the question in investigations on ventricular alternation. I awakened in bloodless frogs' hearts alternation of the ventricle (1915), (1916), (1921) by warming the sinus venosus. During the small ventricle-systoles the apex of the ventricle did not contract, whilst during the big alternation systoles the whole ventricle contracted. By this procedure I was able to obtain complete electrograms of the ventricle during the big alternation systoles and the basal components during the small alternation systoles. Still more remarkable were the results which I obtained when the alternation was developed by poisoning the frog's heart with digitalis or antiarin (1918), (1919), (1921). These experiments were done with the heart in situ and the circulation intact. The digitalis or antiarin were injected subcutaneously. At a certain stage of the poisoning the ventricle-alternation developed. In such a preparation, during the small alternation systoles, a small hernia-like protrusion appeared which was red-colored. This protrusion was usually seen near the apex. It occurred because during the small alternation systoles the apical part of the ventricle did

not contract. The contracting part of the ventricle pressed the blood toward the inactive apex causing the appearance of a red hernia-like protrusion in sharp contrast with the whitish color of the part in active contraction.

In figure 1 an example of such a ventricle-alternation is given which was obtained after poisoning with antiarin. We see in this figure during the big alternation systoles complete ventricle electrograms with R. and T. deflections, whilst during the small alternation systoles the basal component alone appears. It is quite clear here that the ventricular electrogram arises through interference of the basal and apical components, as shown in the diagram, figure 2. The R. and T. deflection of the big alternation systole are caused here through interference of both components, which are indicated under the small alternation systole. The contraction wave starts from the auricle, travels to the base and continues thence over the

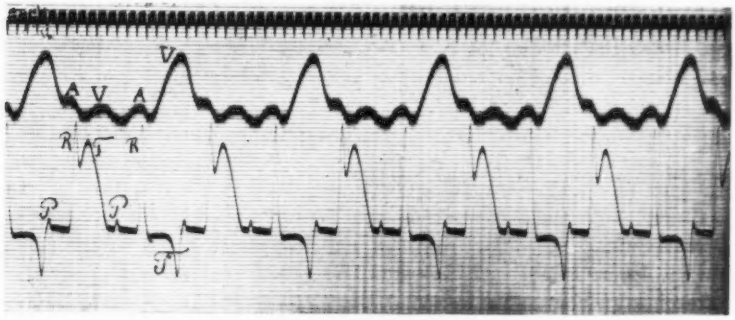

Fig. 1

apex. The basal component *a-b-c* begins sooner than the apical *c-f-g* which causes a deflection in the opposite direction. The basal component lasts as long as the ventricle remains contracting. The apical component begins a little later and brings the initial upward deflection back to the zero. Then both components are in equilibrium and the curve remains in the zero. At the end the basal component lasts in our case a little longer than the apical (or the basal one predominates at the end over the apical) which causes the resultant T-deflection. During the small alternation systole only the base contracts giving the basal component *a-b-c*. In this case the apical component *c-f-g* is absent because the apex is inactive. This alternation of large and small systoles caused by digitalis or antiarin was of very practical assistance because I could thus easily observe with the naked eye that part of the ventricle which was inactive during the small alternation systole (in all figures time in $\frac{1}{5}$ second).

This direct observation of ventricular activity was of the greatest help

in analyzing the electrogram and in following the contraction wave through the ventricle. The hernia-like protrusion usually occurred at the apex. When occasionally it occurred at the base it was always accompanied by a contraction of the neighboring basal tissue. During the small alternation systoles the ventricle never remained inactive along the whole atrio-ventricular groove. Contraction of the apex whilst the base remained inactive never occurred. This evidence indicates that the contraction wave travels from the atrio-ventricular groove through the ventricle in the direction of the apex. The following observation is also of great importance in this connection.

In some cases I could observe with the naked eye that the small alternation systoles were very small and that the hernia-like protrusion took up nearly the whole ventricle leaving only a small ring of the ventricle muscle along the atrio-ventricular groove contrasted white, because it contracted. This indicates that the contraction-wave has worked itself forward into the ventricle muscle during these small alternation systoles along the whole atrio-ventricular groove for a short distance. In such cases the electrograms showed during the small alternation systoles a small basal contraction. From these facts I have to draw my conclusions that in the frog's heart the contraction wave travels along the whole atrio-ventricular groove from the auricles to the ventricle and that it spreads through the ventricle from base to apex. I found a confirmation of this conception in other experiments. When I stimulated the base of the ventricle the resulting electrograms did not differ from the ventricle electrograms of the normal beats (de Boer, 1918). Th. Lewis (1917), (1922) has recently opposed the interferential theory. He writes:

> In this early work the heart was examined by placing on the base and the apex of the ventricle two contacts and connecting them to a galvanometer. Because in such curves as were regarded to be typical, the first deflection indicated relative negativity of the base, it was concluded that the base first becomes active; it was further concluded that the excitation wave spreads as a simple wave from base to apex. Now, further and closer observation by modern methods has shown that the first of these conclusions is usually incorrect and that the last conclusion is *never true*.

And further:

> The excitation first reaches the surface of the heart at neither base nor apex, it reaches the central portions of the ventricle and races simultaneously up to the base and down to the apex; the race may be won in one or the other direction; usually it is won at the apex.

He here disposes of the opinion of the early adherents of the interferential theory, that is to say, that electro-negativity in the basal part of the ventricle is indicated by electro-negativity of the basal electrode and that activity in the apical part of the ventricle muscle is indicated as an

electro-negativity of the apical electrode. Lewis expresses himself as follows:

> These assumptions, erroneous as I believe them to be, arise chiefly from the idea that if muscle at the base is active, the basal contact will show relative negativity and, conversely, that if the apex is active, the apical contact will show relative negativity.

Th. Lewis entirely drops the view that one measures the potential differences between the basal area and that of the apex. Instead of this he assumes that the string-galvanometer registers the direction of the excitation wave. According to this opinion, the points on the surface, in the middle of the ventricle, become first electronegative, and afterwards the points of the apex of the ventricle and the base. This assumption is based on experimental results in which the action currents are led off from the heart surface, a technical procedure which I and others have criticized (1915), so that here I need not come back to the subject.

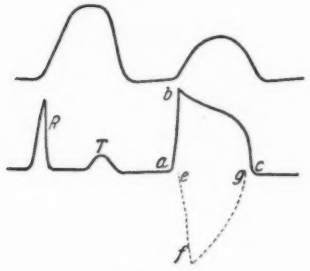

Fig. 2

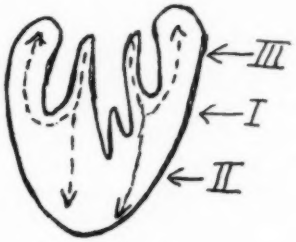

Fig. 3

All of Lewis' conclusions, as well as his circus-movement theory, rest upon this single method of attacking the problem. I have already shown that his conception of auricular fibrillation, based upon the same technique, is incorrect. I shall now consider in detail Lewis' ideas concerning the initial deflections of the ventricle-electrogram.

In the scheme of figure 3 this theory is full explained. The excitation wave moves first in the direction of the apex. This causes the R-deflection. In the meantime the excitation wave moves back in the direction of the base. Lewis writes:

> Activity in the region of the apex still tends to maintain the electric axis from base to apex; the basal activity tends to set it in a contrary direction. But the effect of this basal activity is apparently insufficient to upset the average direction, which still remains from base to apex. The basal contact is throughout relatively negative to the apical contact. If, however, as often happens in the amphibian heart the basal segments of muscle are the best supplied, this opposition of the apical is removed and

the effect of basal activity then appears. It manifests itself in the form of an S wave, a downward deflection indicating relative negativity of the apical contact. The axis of the electromotive force is now from below upward. Thus, our hypothesis brings us to a reasonable explanation of a curious but actual observation, namely, that the appearance of an S wave in the electrocardiogram of amphibians is associated with late arrival of the excitation wave at the ventricular base.

During the R-deflection the excitation wave, according to Lewis, moves downward through the point of the ventricle and during the S-deflection through the base upward. According to Lewis, basal negativity can cause a downward deflection and apical negativity an upward deflection. Only the direction in which the negative wave moves determines the direction of the deflection. According to Lewis, this hypothesis is not contrary to any of the known facts.

I believe this hypothesis to be wholly incorrect and hold that the character of the deflection is determined by the relative conditions under the electrodes. Another hypothesis, according to which the excitation starts at the surface in the middle of the frog's ventricle and travels first to apex and then to base, must also be considered.

We shall first treat the question whether the direction of a deflection of the ventricle electrogram is determined by the direction in which the excitation wave travels through the ventricle; and afterwards we shall see whether the excitation wave in the frog's heart coming from the auricle, arrives at the surface of the ventricle at the centre and races from there up to the base and down to the apex.

In order to get an answer on the first question I made transverse incisions in the frog's ventricle and registered the electrograms. Four types of experiments were performed. In the first group an incision was made through the middle of the ventricle leaving a very narrow muscle-bridge between the base and the apex. (In figure 3 the level of the incision is indicated by *I*.) In figure 4 the electrograms are shown before and after making the incision. On the top line we see the suspension curves and the electrograms of the intact frog's heart. On the second we find the curves after the incision has been made. Note the diagram at the side of this line showing the character of the incision with an arrow to indicate the direction in which the excitation wave must travel. Immediately after making the incision only the base pulsated and electrograms arose, the form of which agreed with the deflections *n-o-r* which occur on the second line alternately. During the registration on the second line curves, every second basal contraction was followed by a contraction of the apex. The deflection *n-o-r* is the electrical equivalent of the base-contraction. If the contraction of the base is followed by a contraction of the apex the electrogram-curve *a-b-s-c-d-e-m* arises. Here we see first an upward deflection, entirely agreeing with the beginning of the base-curve *n-o-r*. At *s* the curve line suddenly descends with a sharp deflection to *c-d*. At this

moment the contraction wave passes the thin muscle-bridge and travels to the apex. The deflection *c-d-e-m* represents the electrogram curve of the apex. In the curve from *d* to *c* a segment is absent, due to the continuance of negativity at the base (heel of Samojloff). We thus see that the negativity of the base of the ventricle gives an upward deflection and a negativity of the apex gives a downward deflection. The direction of both the deflections is determined by the change of condition in the muscle near the two leading off electrodes. It is obvious that, after passing the muscle bridge, the contraction-wave will have a general direction from the base toward the apex (see fig. 4). According to Lewis, an

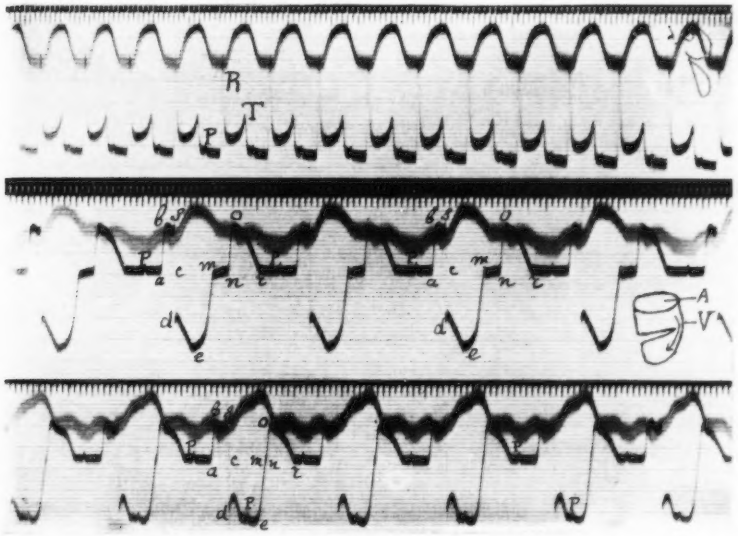

Fig. 4

upward deflection arises when the contraction wave travels in the direction of the apex. With this experiment the incorrectness of this theory for the direct leading off has been shown. The direction of the deflection is here not determined by the direction of the contraction wave, but by the change of condition on both the leading off poles.

The bottom line of curves was registered 5 minutes after the middle line. The contraction wave now travels more slowly from base to apex because the distance from *b* to *s* is here longer than in the middle line. The condition of contraction of the base had lasted longer this time when the negativity of the apex began to dominate that of the base.

In the second group the transverse-incision was made through the

apex so that a small part of the apex was still connected with the ventricle by a narrow muscle-bridge. The level of the incision is indicated in figure 3 by *II*. After making the incision five electrograms were registered. A part of each is shown in figure 5. Five minutes elapsed between all the records except between the third and fourth, when ten minutes elapsed.

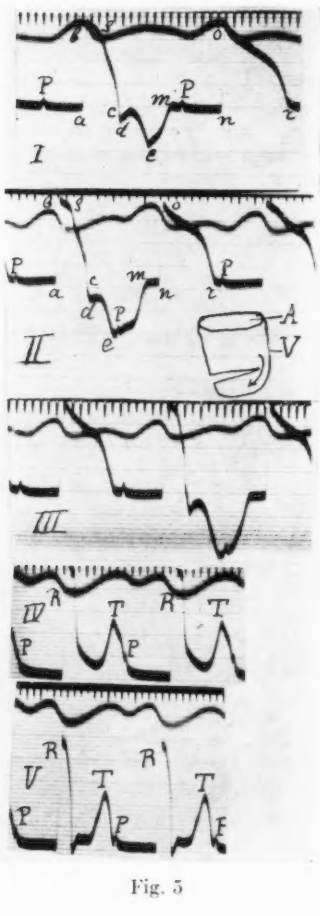

Fig. 5

These records prove clearly that an upward deflection occurs only when the base is negative, and a downward deflection only when the apex is negative. If we indicate the incision in the scheme of Lewis, we shall see that in the upper part of the ventricle the contraction waves, moving toward the apex, are considerably reduced. However, Lewis' point of view for the indirect leading off is that the upward deflection, that is to say, the upstroke of the R-deflection, arises because the moving of the contraction wave in the direction of the apex dominates over that in the direction of the base. He expresses himself as follows:

> The excitation first reaches the surface of the heart at neither base nor apex, it reaches the central portions of the ventricle and races simultaneously up to the base and down to the apex; the race may be won in one or the other direction; usually it is won at the apex.

But in the present experiment the greater part of the contraction waves, which move in the direction of the apex, is absent during a contraction of the basal part. It would therefore be expected, according to Lewis, that "the race may be won at the base" and that a downward deflection occurs during the basal contraction. But this does not happen, as can be seen in figure 5. Instead there is an upward deflection at this time. In row *IV* and *V* the T-deflection is again positive (upward deflection). Now the velocity of the excitation wave along the narrow muscle has again increased, the R-deflection has become narrower. (For literature on the influence of the velocity of the excitation wave on the form of the electrogram of the ventricle, see de Boer, 1918.) This is because at the end the basal

negativity preponderates over the apical and therefore the T-deflection is positive. It follows, therefore, that my second group of experiments also disproves Lewis'[1] theory.

The third series of experiments is also important in the same connection. In these the incision was made transversely over the base of the ventricle near the a-v groove, as shown in figure 3 by *III*.

Since of each pair of basal contractions one is followed by a contraction of the apex, it follows that the basal contraction does not arise from the central portion of the ventricle. (See fig. 6.)

Hence it is justifiable to suppose that the contraction-wave spreads from the a-v groove over the basal part. The contraction of the apex causes a downward deflection in the electrogram although the general direction of the contraction-wave is from base to apex. In the experiments of the fourth group the incision in the a-v groove was made in such a way that the ventricle remained intact. The auricles were now connected with the ventricle by means of a narrow muscle-bridge. The ventricular

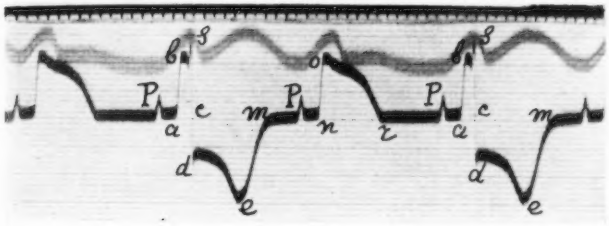

Fig. 6

electrogram did not show any essential difference from the normal. In figure 7 we see close together: first the electrogram before the incision was made, then immediately after the incision and then 5 minutes later.

Some ten experiments were performed in this manner, all of which gave similar results. In all experiments the electrograms were led off by placing the non-polarizable electrodes, one on the auricles as low as possible, and one on the apex of the ventricle as high as possible. Both electrodes were applied at the dorsal side of the heart.

All these four groups of experiments have shown that the direction of the deflections of the ventricle electrogram is not determined by the direction in which the contraction-wave spreads through the ventricle, as indicated by Lewis. On the contrary, they all support the interferential theory.

Now we shall consider the other question, namely, where the excitation-wave enters the ventricle. We have already pointed out that in the experi-

[1] Ergebn. d. Physiol., Bd. xxi, Abt. A, pp. 93—113, München Verlag von J. F. Bergmann, 1923.

ments, in which the incision was made through the base of the ventricle near the a-v groove, that portion of the base between the incision and the a-v groove contracted due to an excitation-wave originating in the auricles. The contraction of the base could not start now from the centre of the ventricle because the incision has been made between the centre and the a-v groove. This observation shows that the base can contract directly as the result of an impulse starting in the auricles. There is no reason why this should not happen normally. Furthermore, the deflection of the electrogram of this base contraction is in the same direction as that of the R-deflection of the normal electrogram of the ventricle. If after about half an hour's waiting the conduction along the muscle-bridge has improved the basal and apical components appear again more simultaneously and a normal electrogram of the ventricle occurs with an R- and a T-deflection in the same direction as the P-deflection. (See also fig. 5, *IV* and *V*.) In the experiments in which the incision near the a-v groove is through the base, a normal electrogram of the ventricle arises and we know in this

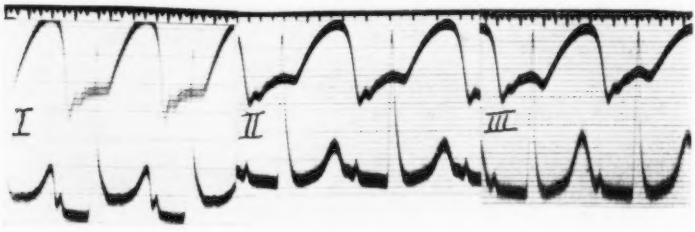

Fig. 7

case with certainty that the excitation-wave has reached the base of the ventricle directly from the auricles.

I have made another observation which is of great importance for our problem. When we poison a frog's heart with digitalis or antiarin, very often an alternation of the ventricle arises. During the small alternation systole generally a part of the point of the ventricle protrudes as has been described. This hernia-like protrusion develops because this area of the ventricle does not contract during the small alternation systole and is forced out by the remaining area which does contract. Several instances occurred in which alternation systoles were very small. I could then observe that nearly the whole ventricle expanded and was filled with blood; only a small ringlike strip of the ventricle muscle along the atrio-ventricular groove was white colored and contracted. The protrusion was here very large and occupied nearly the whole ventricle: only a small ring along the a-v groove was contracting. This observation shows clearly that the excitation wave enters the ventricle along the whole a-v groove. The results

obtained with the incision experiments show the same thing, that is, that the excitation-waves, starting from the a-v groove, spread to the apex.

I should like to discuss one more question. In my theory of fibrillation I presented a new idea in the physiology of the heart. If a portion only of the ventricle is in contraction, the remaining portion of the ventricle may still be refractory.[2] If now a short time afterwards the refractory period ends, the excitation wave can move on further from the contracting portion. We know that the muscle of the ventricle remains for a long time in condition of contraction. Herein the muscle of the ventricle differs from skeletal muscle. In the latter muscle each part remains in a state of stress only for a very short time during a single contraction (twitch). In the ventricle, however, the contraction in each part lasts considerably longer. The excitation wave can spread during contraction. This is an essential part of my fibrillation theory and the observations established it with complete certainty. In the experiments with ventricular incisions the base contracted following auricular activity. From the contracting base the excitation wave travelled along the muscle-bridge toward the apex. The contracting base can, however, discharge an excitation wave to the apex at different times during the contraction.

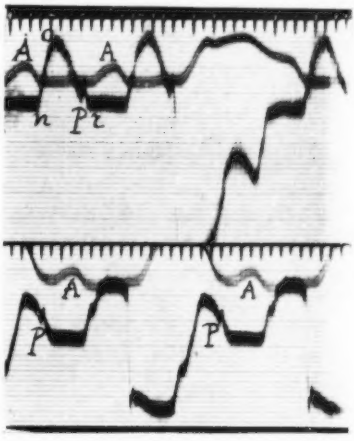

Fig. 8

We can see from the electrograms of figures 4, 5 and 6, that at different times in the beginning of the base-contraction, the excitation wave can travel further to the apex of the ventricle. But also toward the end of the contraction of the base, the excitation wave can spread further to the apex. Figure 8 is an example. In this instance a basal incision had been made and the electrograms were made immediately afterwards. At first a small portion of the base contracted after each contraction of the auricle, and after about seven base contractions, a contraction of the apex also followed. On the top line of figure 8 we see the electrograms of three of these base-contractions, *n-o-r*. The second one was followed by a contraction of the apex but the first was without effect. Toward the end of this second basal electrogram, the curve suddenly deflects downward sharply. At this moment, that is to say, at the end of basal activity, an excitation wave

[2] See S. de Boer: Die Physiologie und Pharmakologie des Flimmerns, p. 76-83, München, J. F. Bergmann, 1923, and Ergebnisse der Physiologie, Bd. xxi, Abt. 1.

reaches the apex, over the muscle-bridge, which then begins to contract. Five minutes later the conduction has improved and every second base contraction is followed by a contraction of the apex. Now at a much earlier period of the base contraction the excitation wave travels to the apex and causes it to contract. The electrogram of the next base-contraction following apical activity is smaller because the apical activity has not ceased and therefore the forces are opposed. We may therefore positively conclude that an impulse can spread from an area of activity at any time during the contractile phase. This lends secure support for my theory of fibrillation.

In the preceding pages I have shown that the excitation-wave is not propagated through the ventricle of the frog's heart, as understood by Lewis. Lewis' procedure was to place one electrode on the ventricle and the other on the chest wall. The resultant records showed both intrinsic and extrinsic deflections. Lewis assumed that the biggest deflection was intrinsic. He does not explain the justice of this assumption, saying only that, "we are confident it represents the intrinsic deflection" and then he continues, "Where the curve is of less distinctive form, its form may be altered oftentimes by my moving the distal (or chest wall) contact either to another point on the chest wall or on to the heart itself." We see therefore that this method is unreliable and that it can not fix the instant of passage of an excitation-wave over a given point on the ventricular wall. Hence the far-reaching conclusions which he draws are of no value. I have already shown that his conclusions are inapplicable to the direction in which the impulse travels in the frog's heart. Lewis' final conclusion is that the excitation wave, after passage over the His-Tawara bundle, is quickly propagated over the Purkinje fibres and then more slowly through the ventricular muscle. He estimates roughly that the velocity of the excitation wave from the inner to the outer surface or along the outer surface is one-tenth of the velocity with which it travels over the Purkinje fibres. I am convinced that such a deduction is wholly unwarranted by Lewis' experiments, and that the results which he obtained are due to abnormal experimental conditions. The heart was exposed under artificial respiration and its outer surface necessarily cooled while the temperature of the inner surface was maintained by the circulating blood. These different temperature conditions are bound to cause untrustworthy results for the relative velocity in the Purkinje system.

I shall now discuss some of the particular procedures followed by Lewis. In first instance he makes an incision perpendicular to the outer surface, by which the velocity of the contraction-wave through the ventricle is not delayed. There arises, however, a delay when the incision is applied at the inner surface. This result we could expect. It will be clear that the velocity with which the contraction-wave is propagated through the ventri-

cle is determined by the velocity along the inner surface which has a higher temperature. During normal systoles points on the inner surface of the ventricles are active earlier than points of the outer surface. This is so because the excitation-wave travels along the His-Tawara bundle. Lewis' method of velocity determination is to measure the elapsed time of the excitation wave in passing from the inner to the outer surface.

In other experiments he stimulates a point on the outer surface some distance from the contacts on the inner and opposite outer surface. Under such conditions the endocardium receives the excitation wave before the epicardium but it does so by precisely the same time interval as during the natural heart beat. When he scratches the lining between the point which was stimulated and the leading off electrode on the lining, the point on the lining no more first becomes active. A deep incision in the outer surface did not alter these time-relations. The difference of temperature between the inner and outer surfaces gives a reasonable explanation of such results. The place of greatest velocity for the excitation wave is determined by the

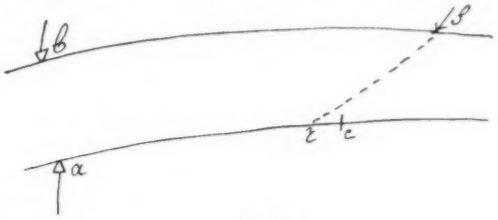

Fig. 9

relative temperature. But I am not wholly clear as to the result of this experiment. If we represent the wall of the ventricle as in figure 9 with the lead electrodes *a* and *b* on the inner and outer surfaces and an incision on the inner wall at *c*, and conceive of a stimulus applied at *S*, we should expect the excitation wave to reach *a* before it reaches *b* if the conduction time is ten times as fast along the Purkinje fibres as it is elsewhere, according to Lewis. The velocity of the excitation wave through the muscle of the ventricle and along the Purkinje tissue, was measured in the following way:

> Again if the rate of conduction between two points on the surface of the heart is examined and these points lie on the wall of the ventricle where this is thickest, the conduction rate is approximately 300–500 mm. per second; a similar rate of propagation is ascertained during the natural passage of the wave from within outwards through the muscle wall, but if the rate of conduction is tested similarly over thin portions of the ventricular wall, it is found to be as high as 1500–2000 mm. per second. In the last-named circumstances the wave is short-circuited through the Purkinje network for the largest part of its course. After consideration of all the circumstances we have concluded that the rate of conduction through ventricular muscle

lies between 300–500 mm. per second, while that through straight paths of Purkinje tissue lies between 3000–5000 mm.

It is clear from these experiments that we may conclude that the velocity at the outer surface is greater where the wall of the ventricle is thin, because the thin wall can be heated from the inner surface easier than the thick wall. Therefore it is also clear that *first those points at the outer surface are activated during the normal systoles* which lie on thin parts of the wall of the ventricle

For the foregoing considerations the experiments which Lewis did with toads and tortoises are very important. These are cold-blooded animals and if my views are correct Lewis should not have found any difference of conduction-time between the Purkinje tissue and the muscle of the ventricle. Here are Lewis' words: "There is in the toad's heart no evidence of specialized conducting system in which the rate of conduction is peculiarly high" and for the tortoise: "The investigation of the tortoise ventricle produces no evidence that there is in this organ any special tissue which conducts more rapidly than the surface muscle-fibre."

With these results before us I believe I am justified in concluding that my explanation of his experiment is correct. In any case I can conclude that the evidence offered to prove *that the network of Purkinje conducts the excitation wave ten times as quickly as the muscle of the ventricle,* is wholly inadequate. (In none of his publications Lewis mentions the temperature factor, which I bring here to the front.)

That moreover it is impossible to determine with certainty at which moment the excitation wave passes a fixed point of the ventricle is clear from the fact that Clement obtained with differential electrodes results which do not agree with those of Lewis. Lewis mentions this in the following words: "The excitation wave does not appear simultaneously over the ventral surface as Clement has stated." The evidence that the velocity of the excitation wave is more rapid in the Purkinje system than it is in the ventricular muscle is, it seems to me, open to serious question. We can not therefore use such evidence to deduce characteristic functional differences in the cardiac tissues.

CONCLUSIONS

The results of this investigation are as follows:

1. In the frog's heart, with leads from an auricle and the ventricular apex, the contraction of the ventricular base causes a deflection of the electrogram *similar* in direction to the P-deflection caused by the auricular contraction, and the contraction of the ventricular apex causes a deflection *opposite* in direction to the P-deflection.

2. The deflections of the ventricular electrogram are determined by change of condition in the tissues beneath the leading off electrodes.

3. The direction of the R and S deflections in the ventricular electrogram of the frog is not determined by the direction of the contraction waves in the sense proposed by Lewis.

4. The contraction wave travels from the a-v groove to the apex.

5. A contracting part of the heart can send out excitation waves at any time during the phase of contraction.

6. During normal systole (with open thorax) the excitation wave reaches the outer surface of the ventricle earlier where the wall is thin than where it is thick. This is due to the shorter distance travelled and (in the mammal) to the fact that the circulating blood better sustains the temperature on the outer surface over the thinner wall.

7. There is no convincing evidence that the Purkinje fibres transmit the excitation wave more rapidly than does the rest of the ventricular mass.

8. The results of Lewis in support of this hypothesis are readily explained by the cooling of the outer surface of the heart incident to exposure and artificial respiration.

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OBSERVATIONS UPON THE CONTRACTIONS OF THE GALL BLADDER

N. B. TAYLOR AND M. J. WILSON

From the Physiological Laboratory, University of Toronto

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The existence of rhythmic contractions of the gall bladder has been known for many years. They were first recorded graphically by Doyon (1893), and the physiological and pharmacological reactions of the viscus have since been investigated by many workers, notably Bainbridge and Dale (1905). The literature on gall bladder function has recently been fully reviewed by Mann (1924).

In dogs under the influence of morphia, Bainbridge and Dale found rhythmic gall bladder contractions at the rate of one to three per minute, which is approximately the same periodicity as peristalsis of the stomach, especially when depressed by the alkaloid. Courtade and Guyon (1904) showed that the motor fibers of the gall badder were derived from the vagus, and coursed along the stomach, the hepatico-duodenal ligament, and the common and cystic ducts.[1] It seemed to us advisable to attempt to record the contractions of the stomach and those of the gall bladder simultaneously, and thus discover whether or not any relation existed between them. When a proper technique had been evolved, a number of other problems were investigated.

METHODS. Experiments were carried out on 55 large dogs and 1 cat. In most cases the animal was fed just prior to the operation with meat, or meat and dog biscuit, as Okada (1915) had shown that the rhythmic activity of the gall bladder was much more marked during digestion than in starvation. In other experiments the dogs were starved for 24 hours. Light ether anesthesia was employed throughout each experiment, assisted by urethane in two animals. Usually a tracheal cannula was inserted. At first we made use of the technique of Bainbridge and Dale, which consists in splitting the sternum, and cutting through both sides of the diaphragm, thus permitting the liver to fall back into the thorax, and freeing the gall bladder so that it is unaffected by any changes in abdominal pressure. To eliminate the effects of engorgement of the liver, the gall bladder

[1] Some doubt has been cast on this conclusion by the recent dissections of McCrea (1924) in the human stomach.

was stripped from the viscus along most of its length. The thorax and abdomen were covered with cloths wrung out of hot saline. The animal was kept alive by artificial respiration.

On account of the profound shock incident to this extensive operative interference, and the difficulty of keeping the viscera at an even temperature, this procedure was replaced by the following:

The abdomen is opened in the midline from the xiphoid process to the umbilicus, or lower. The fundus of the gall bladder is picked up by forceps and the bile evacuated by means of a syringe and large needle. A hole, 1 cm. in diameter, is then made in the fundus, and an empty thin-walled rubber ballon of approximately the same size as that of the gall bladder, tied securely on a small, thick-walled rubber tube, is inserted, and retained in position by a purse-string suture. Tugging on the viscus is carefully avoided in performing these operations. The abdomen is now closed by clamps, and the animal kept warm by suitable measures. In the course of an hour, or at most two hours, the gall bladder will have recovered from the shock, and the balloon is blown up with air, to a pressure of 5 to 15 cm. water, at which it is maintained. The water manometer is connected to a small tambour, which carries a writing point arranged to record on a smoked drum. If no rhythmic activity can be seen in the course of another hour, it is unlikely that it will be subsequently seen, and the animal is useless for the gall bladder experiments except for the investigation of tonus changes. Some methods by which rhythmic contractions can be induced or increased will be discussed later.

The effects of changes in abdominal pressure—principally respiratory—can be almost wholly eliminated by leaving a portion of the wound open when clamping the walls of the incision. The contraction of adjacent viscera has in our experience been negligible. Possible variations of external pressure, due to "changes in volume and turgescence of the liver substance" (Bainbridge and Dale) were overcome by enclosing the gall bladder, stripped from the liver after its contractions in situ had been recorded, in a special glass cylinder, large enough to leave an air space between the gall bladder and its walls, and fitting loosely around the pedicle. A large rubber tube at the top protudes through the abdominal incision. The bleeding which follows stripping the gall bladder from the liver is frequently considerable, there being usually one or two small arteries which give trouble. Possibly this is the reason of our failure in several instances to obtain any rhythmic contractions after "shielding" a previously active organ.

The advantage of this technique is that the glass cylinder almost completely eliminates effects due to swelling of the liver, as well as variations in intra-abdominal pressure. The latter is shown by the great decrease in size of the respiratory variations, and also by the fact that considerable

manipulation of abdominal viscera has little or no effect on the tracing. At the same time some of the disadvantages inherent in the method of Bainbridge and Dale are avoided. The only possible causes of rhythmic pressure variations in our records, other than activity of the gall bladder musculature, are influx and reflux of bile through the cystic duct, and similar

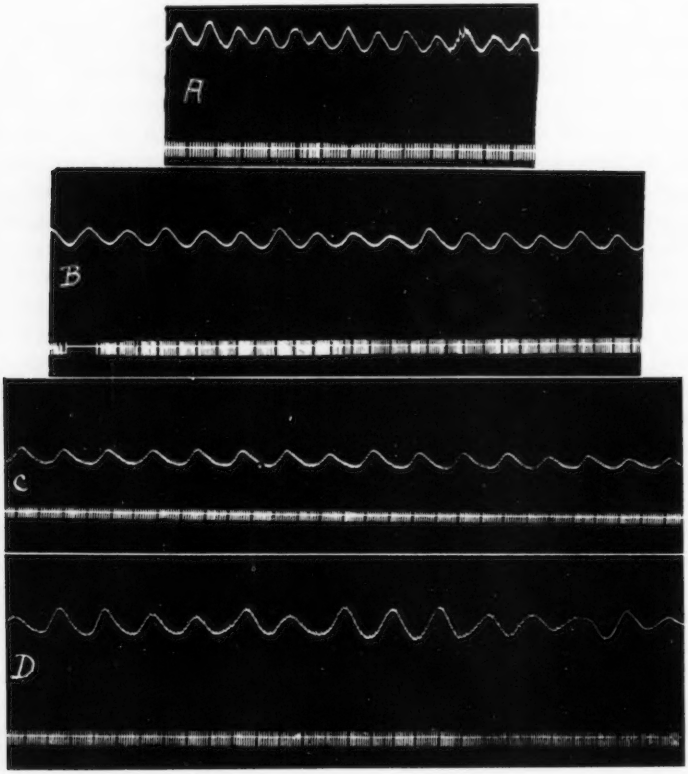

Fig. 1. Examples of normal gall bladder contractions. A and B are from the same dog, A before and B one hour after "shielding." Time marker, 1 second.

changes in blood or lymph pressure. These factors we consider negligible but their possible effect must be admitted. The blood and lymph factors cannot be eliminated without perfusion experiments unless the gall bladder is rendered anemic. Comparison of results by the balloon method with those obtained with strips of the gall bladder seems to indicate that all these possible factors can be disregarded.

Rhythmic activity of the stomach was simultaneously recorded by the

method of Wheelon and Thomas (1922). We used a triple chambered rigid enterograph, made of metal because of the difficulty of stopping leaks in one of hard rubber.

RESULTS. *Normal contractions.* These were obtained in 60 per cent of the dogs, and examples are shown in figure 1. In some cases no contractions at all could be demonstrated, while in others they were present only part of the time. The factors concerned in the appearance or disappearance of strong rhythmic contractions of the gall bladder were carefully studied. In many cases periods of marked activity and comparative quiescence succeeded each other at intervals of about one minute. Deep anesthesia abolished all contractions. Lengthening the respiratory dead space was held responsible for the disappearance of the contractions in some instances, chiefly because of their analogy to some experiments on human stomachs, in which it was found that gastric peristalsis in man could be abolished by breathing in and out of a rubber bag filled with oxygen (Dickson and Wilson, 1924).

The movements were found to be extremely susceptible to various conditions. For example, if the gall bladder became chilled the contractions were abolished, or, stripping the gall bladder from the liver not infrequently resulted in cessation of previously well-marked activity. This may have been due to vascular interference, or possibly because of injury to nervous structures. Evidence of a relationship between the gall bladder and duodenum was obtained from the observation that any manipulation of the duodenum was almost invariably followed by a disappearance of the rhythmic contractions of the gall bladder. We formed the impression that the breed of the animal may have had some influence, as our best results were obtained from dogs showing marked characteristics of Airedale or Irish terrier. The best animals were large ones, weighing from 15 to 25 kilos. The age of the dog had probably some influence, although we cannot be definite on this point. Sometimes adrenalin intravenously was followed by marked contractions. Gall bladders containing clear golden yellow bile usually gave better contractions than those with dark turbid fluid. No gall stones were found, but in some cases the bile was almost granular in appearance, and was withdrawn through the needle with some difficulty.

Though the operation necessary for "shielding" usually caused the amplitude of the contractions to be decreased, it often increased the rate of contractions. The rate, however, showed considerable variation from hour to hour in each dog, in one animal a complete contraction varying from 14 to 28 seconds at different periods. In general, the rhythm was slow at first, then became faster, and finally slowed as the animal became moribund. In all our tracings, the shortest time for a complete contraction was 10 seconds, and the longest 32 seconds. These results are in the absence of drugs, other than the anesthetic.

One experiment was performed on a cat, but the gall bladder was found to be too small for our purpose. No contractions could be observed.

Gall bladder and stomach contractions. Presumably for one or more of the reasons outlined above, the contractions in many dogs in which simultaneous tracings of stomach and gall bladder activity were attempted proved very unsatisfactory. Some of the better results are shown in figure 2. In every case in which definite rhythmic contractions of the gall bladder were obtained, no time relation between their contractions and those of the stomach could be made out. A careful examination of all our records shows that in some cases the gall bladder contractions are

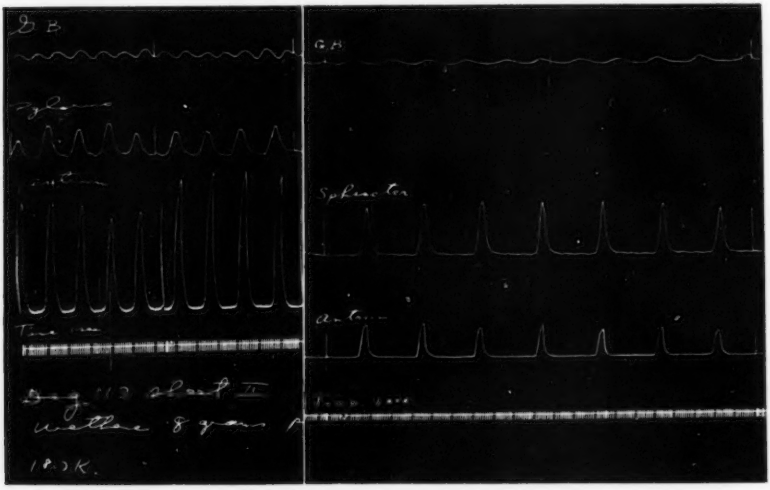

Fig. 2. Contractions of gall bladder, pyloric sphincter, and pyloric action recorded simultaneously. Time, 1 second.

faster than those of the stomach, in other cases slower, and that the former cannot be ascribed to "blocks" in the conduction of gastric peristalsis.

Epinephrin. The intravenous injection of 0.5 cc. adrenalin chloride 1/1000 (Parke Davis & Co.) did not have constant effects in the unshielded gall bladder, but resulted in a definite series of changes when the viscus was separated from the liver and enclosed in the glass bulb. The first effect was usually a slight rise in pressure, followed by a profound fall which was succeeded by a rise, frequently to a level higher than the initial one, with a marked increase in the amplitude of the rhythmic contractions (fig. 3).

The effect of repeated injections of 0.5 cc. adrenalin 1/1000 is shown in figure 4. It will be seen that the initial fall in pressure is abolished at the third or fourth injection, but that the tonic effect increases. Morphine and

apomorphine tend to eliminate the initial fall after adrenalin, with a return to the original picture after the animal has recovered from the depression of the narcotic. Control experiments with chloretone (used as a preservative in the Parke Davis preparation) were not attempted.

EFFECT OF SUBSTANCES IN THE DUODENUM. *N/10 hydrochloric acid.* The introduction of 1 to 10 cc. N/10 HCl into the duodenum usually caused a rise in tone of the "shielded" gall bladder, though it had little effect on the rhythmic contractions. Water at 12° to 39°C. had no effect in quantities of 10 to 40 cc. Thirty per cent solutions of sodium chloride had no influence.

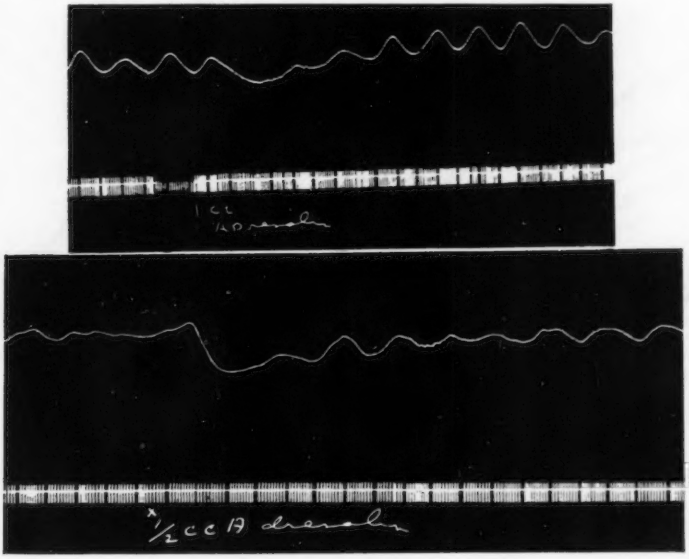

Fig. 3. Effect of adrenalin (intravenously) on contractions and tone of "shielded" gall bladder. Time, 1 second.

Magnesium sulphate solutions. In view of the controversy raging around the so-called Meltzer-Lyon test, we made a number of experiments in an endeavor to find whether or not a dog's gall bladder contracted when strong solutions of this salt were introduced into the duodenum. This test, it will be recalled, is a clinical one, and consists in introducing a quantity of magnesium sulphate solution directly into the duodenum, and subsequently aspirating the contents. One portion of the fluid obtained contains darker bile than the rest, and is believed by many clinicians to be bile expressed reflexly from the gall bladder.

This work was commenced before the publication of the paper of Matsuo (1924), who actually saw the gall bladder contract in two human subjects whose abdomens had been opened under local anesthesia. The contractions occurred about 5 minutes after giving the $MgSO_4$ solution.

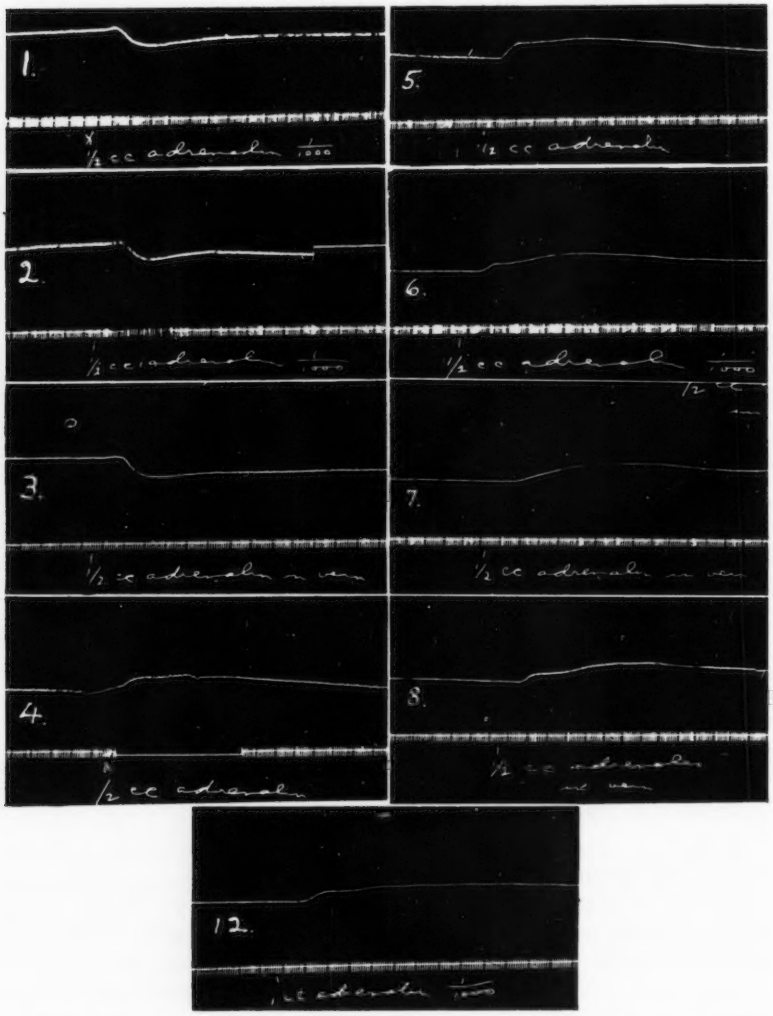

Fig. 4. Effect of the repeated intravenous injection of adrenalin on gall bladder tone. Digestion at intervals of 10 to 15 minutes gall bladder "shielded." Time, 1 second.

In our observations the gall bladder was not "shielded," and a soft rubber catheter was introduced through the anterior aspect of the stomach and passed through the pylorus, the tip being 2 finger-breadths into the duodenum. This was effected with a minimum of disturbance by a glass tube of appropriate size. Forty cubic centimeters of 33 per cent $MgSO_4$ solution were warmed to 39°C. and introduced into the duodenum.

In 9 experiments with this technique on 8 dogs, 4 gave slight tonic increases (fig. 5B) and one (dog 150) showed a strong contraction after a lapse of 4 minutes (fig. 5A). The latter result is difficult to explain in any way other than as a contraction resulting from the $MgSO_4$ administration.

Dog 150 is further distinguished by the fact that he exhibited the magnesium sulphate anesthesia described by Meltzer. He received at intervals

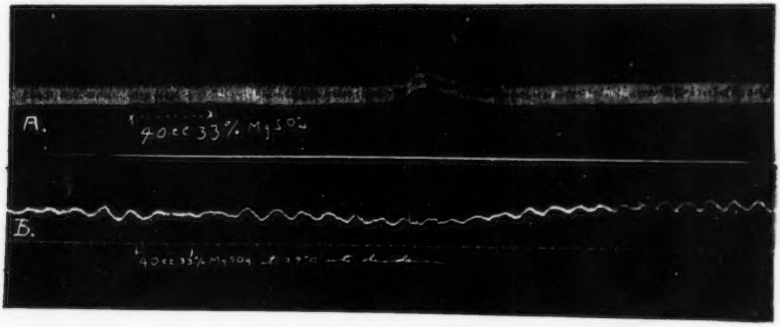

Fig. 5. Effect on gall bladder tone of concentrated solutions of $MgSO_4$ introduced into duodenum. Not "shielded." Upper tracing (dog 150) time by watch approximately 4 minutes from commencement of injection until reaction appeared. Lower tracing—time, 10 seconds.

4 injections of 40 cc. 33 per cent $MgSO_4$ into the duodenum, and after the second injection was perfectly relaxed, with regular pulse and respiration, and loss of corneal reflex. He remained so for 2 hours without any other anesthetic.

The tracing in figure 5B was obtained on the first injection.

Only two other dogs showed loss of the corneal reflex, and both required ether continuously to maintain anesthesia. Obviously the absorption of solutions of magnesium varies with the individual.

Other results. Intravenous injection of 2 or 3 cc. of the animal's own bile (after standing in air for an hour or two) lowered the tone and depressed the rhythmic activity. Smaller quantities of bile sometimes had no effect. Five-tenths cubic centimeter 1 per cent pilocarpine nitrate solution raised the tone slightly, but had no effect on rhythmic movements. These results agree with those of Bainbridge and Dale (1905).

Morphine intravenously caused no evident initial increase of rhythmic activity, such as has sometimes been shown on the stomach. After a time it definitely slowed the rhythm, with little effect on the amplitude. Apomorphine had a similar effect. Secretin intravenously had no appreciable influence on tone or rhythm.

Eight to 12 cc. of 30 per cent NaCl solution intravenously caused slightly increased contractions of the gall bladder in two dogs. Usually there was no effect on the tone, but on two occasions it fell appreciably. This experiment was suggested by the observations of Hughson and Scarff (1924) on increased intestinal peristalsis following the intravenous administration of this substance in cats.

SUMMARY

1. A method is described for recording contractions of the gall bladder in dogs which eliminates some defects.

2. Rhythmic contractions of the gall bladder can be demonstrated in 60 per cent of large dogs. These contractions bear no time relation to gastric peristalsis.

3. A marked contraction of the gall bladder on injecting 40 cc. of 33 per cent $MgSO_4$ solution into the duodenum was found in one dog out of eight; slight tonic contractions were seen in several others.

4. Epinephrin intravenously caused a fall in gall bladder pressure, succeeded by a marked rise in pressure and increased amplitude of the rhythmic contractions. Repeated injections abolish the initial fall, but increase the tonic effect.

5. Pilocarpine raises the tone slightly, but has no effect on rhythmic activity.

6. Bile (3 cc.) intravenously lowers tone and depresses the rhythmic contractions.

Morphine and apomorphine slow the rhythm, without affecting the amplitude. Secretin intravenously has no appreciable effect on the motor activity.

7. Ten cubic centimeters 30 per cent NaCl intravenously temporarily increased the rhythmic contractions.

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TONUS RHYTHMS IN THE BOWEL

WALTER C. ALVAREZ

From the George Williams Hooper Foundation for Medical Research, University of California Medical School, San Francisco

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Most writers on the mechanics of digestion speak of two types of activity in the small bowel—the rhythmic segmenting movements and the peristaltic rushes. It is not so generally known that there are, in addition, rhythmic tonus changes, changes which probably have a good deal to do with the progress of events in the digestive tract. I have been studying and recording these waves for the last twelve years but only recently, with a better technic, have I been able to show that, especially in the cat, they may appear alone without any superimposed small contractions. See figure 1.

The animals—cats and rabbits—are anesthetized with urethane (2 grams per kilo by stomach tube); the cord is pithed from the lumbar region up to a point between the scapulae; the abdomen is opened under a bath of warm Locke's solution; points on the bowel are immobilized in the grip of little wire clips fastened to L rods, and the contractions of the circular or longitudinal muscles (as desired) are recorded on a smoked drum with the help of threads running to light heart levers.

With the help of this technic Miss Mahoney and I showed two years ago (1923) that there is a tonus rhythm in the duodenum of the rabbit which ordinarily has about the same rate as that of the gastric waves, and which therefore is often captured by the stomach and made to follow it for minutes at a time. Wheelon and Thomas (1922) observed what is probably the same phenomenon in the dog. In the cat this tonus rhythm has a rate of from 1.5 to 3.5 per minute while the gastric waves run usually about 5 per minute. The independence of the two rhythmic activities is well seen in figure 1 A where for part of the time the antral muscle was plainly at rest. (The smallest contractions in all the records are respiratory in origin.) Furthermore, with a hand lens it can be seen in this record that, occasionally, while the duodenum was contracting 3.3 times per minute, the antrum was contracting 11 times. Sometimes, as in figure 1 B, we get the impression that the tendency to contract with a tonus rhythm has spread upwards from the lower bowel to the stomach. This takes place very definitely after the colon of the animal has been stimulated by the injection of soapy water.

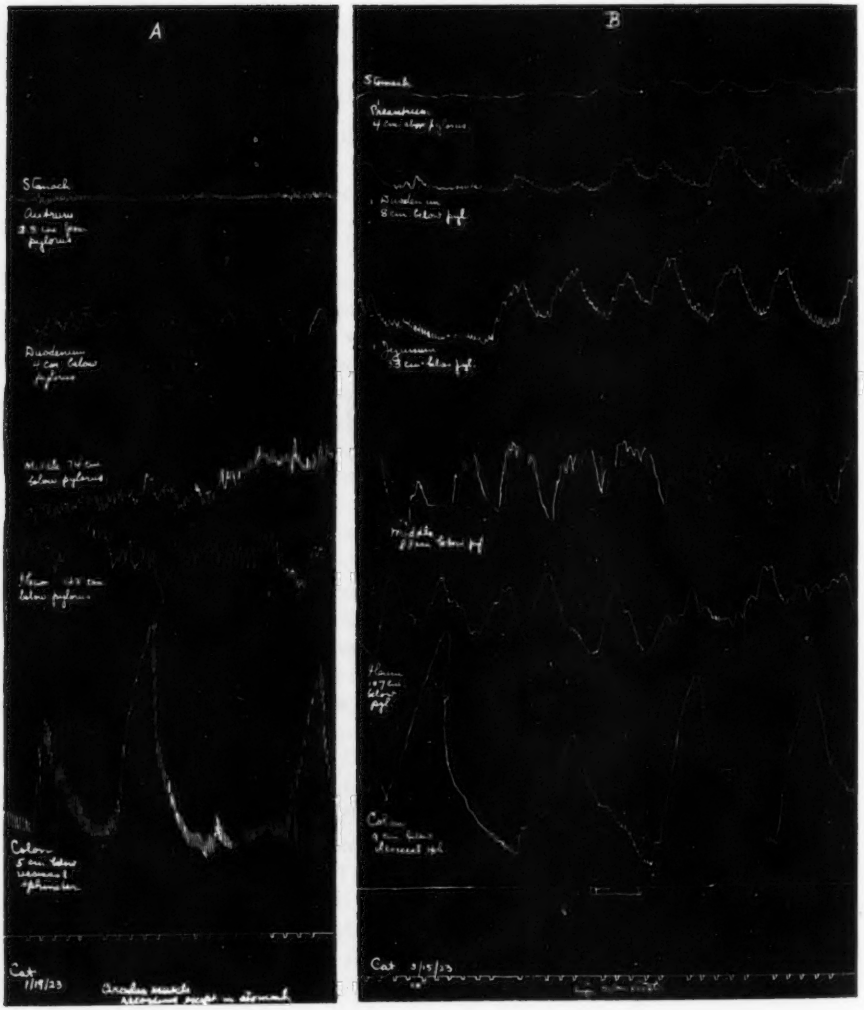

Fig. 1. Records from six different parts of the digestive tract of the cat opened under salt solution. Read from left to right. The writing lever magnified four times.

So far as I can see, the tonus waves ordinarily do not travel along the bowel. It is possible that they do so in cases of intestinal obstruction where I have found a slowly progressing type of wave different from that observed in health.

The fact that these tonus rhythms appear when the segments are contracting outside of the body in oxygenated Locke's solution shows that they can arise in the wall of the gut itself. Figure 2, A and B, shows how they appear in the records from excised pieces of rabbit's small bowel. In this case the rate is about 1 per minute or slower than that observed ordinarily in the intact animal. Similar tonus waves are prominent features also of the records made from the excised colon of the rabbit or from the small bowel of the cat or white rat. In fact, the appearance of tonus waves is common in the records from many types of smooth muscle, and even in those from skeletal muscle, when it is stimulated rhythmically.

Figure 2 C shows the tonus contractions in the jejunum of a man who allowed me to pass a small balloon into his bowel through a fistula made a short distance below the ligament of Treitz. This fistula had been made a month or two before in the hope of healing an ulcer near the cardia. Inasmuch as an operation done a few weeks later showed that the ulcer had healed completely, I can say that at the time of the experiment I was dealing with a pretty normal man.

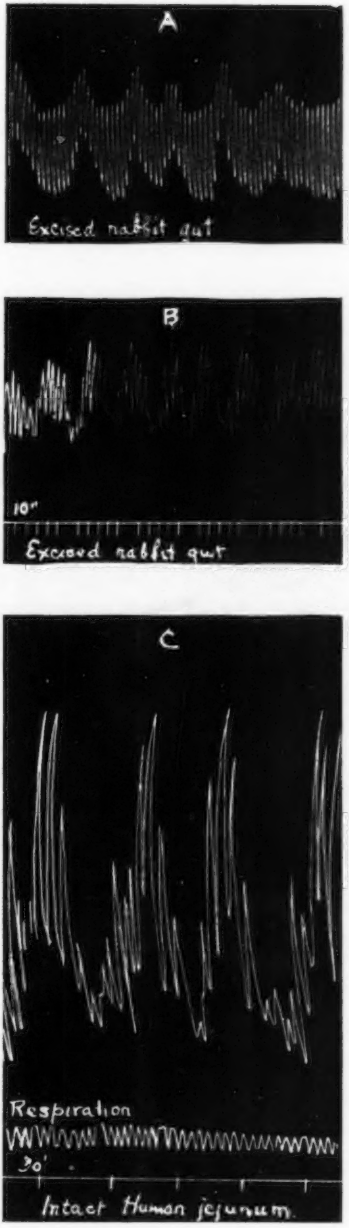

Fig. 2 A and B. Records from the excised small bowels of rabbits. C, record secured from a man with a jejunal fistula.

It is well known that with certain types of smooth muscle an increase in the tension will bring about an increase in the rate of rhythmic contraction (Straub, 1904). In 1915 I spent a few months trying to show this with intestinal segments but never reported the work because the results were not clear-cut. Distention or weighting often failed to produce any change, and when there was an increase in rate, it generally remained or became more marked when the tension was removed. Similar negative results were obtained by Magnus (1904) and by Bottazzi (1898). Our failure was probably to be expected, however, because as v. Uexkull (1904) has pointed out, a stretched muscle *loses* its tone. What we should have tried to get was a contracted muscle. Bottazzi (1899) got it by stimulating the esophagus of the toad electrically, and he did succeed in increasing the rate of the rhythmic contractions. With the new recording technic we can now see what happens to the rate of the rhythmic contractions when the tone of the bowel increases spontaneously, and we do find that sometimes —not always—the rate follows the tone up and down. In figure 3 A the rates were 13–15.4–11.7 per minute. In another they were 12–14.4–11.7, and in another, 13.7–15–13. These observations can probably be linked up with others made by me (1922), by Trendelenburg (1917) and Wolff (1902) which suggest strongly that there is a gradient of tone down the bowel, parallel to the gradient of rhythmicity and perhaps underlying it.

In figure 3 B we see that occasionally a big tonus wave in some part of the bowel will be synchronous with changes in the activity of other parts of the tract. In this case the rise in the lower ileum seems to be associated with a rise in the tone of the jejunum, an increase in the activity of the colon, and a cessation of the activity of the stomach.

Discussion. The question next arises: What is the value or significance of these tonus waves? Unfortunately, we cannot say much about that yet. We know from the work of Cannon (1911) that tone changes can play a large part in determining the points of origin and direction of travel of waves, and if we accept the gradient theory of peristalsis (Alvarez, 1922) it will be obvious that such ups and downs must change the gradient and must therefore modify the progress of material through the bowel. Time and again I have seen the peristaltic rushes start in tonus waves and as often I have seen them stopped by such waves (1924).

It is noteworthy that one bit of smooth muscle can contract in so many different ways at once. It will show the purely local, slow, tonus contractions and the more rapid rhythmic segmentations, and at the same time it will respond to traveling ripples or waves.

A few years ago I was impressed by the fact that the muscle in different parts of the digestive tract is more or less specialized and fitted for the type of work which it has to do. Thus the muscle in the antrum of the stomach seems rather sluggish and tends to contract but three times per minute,

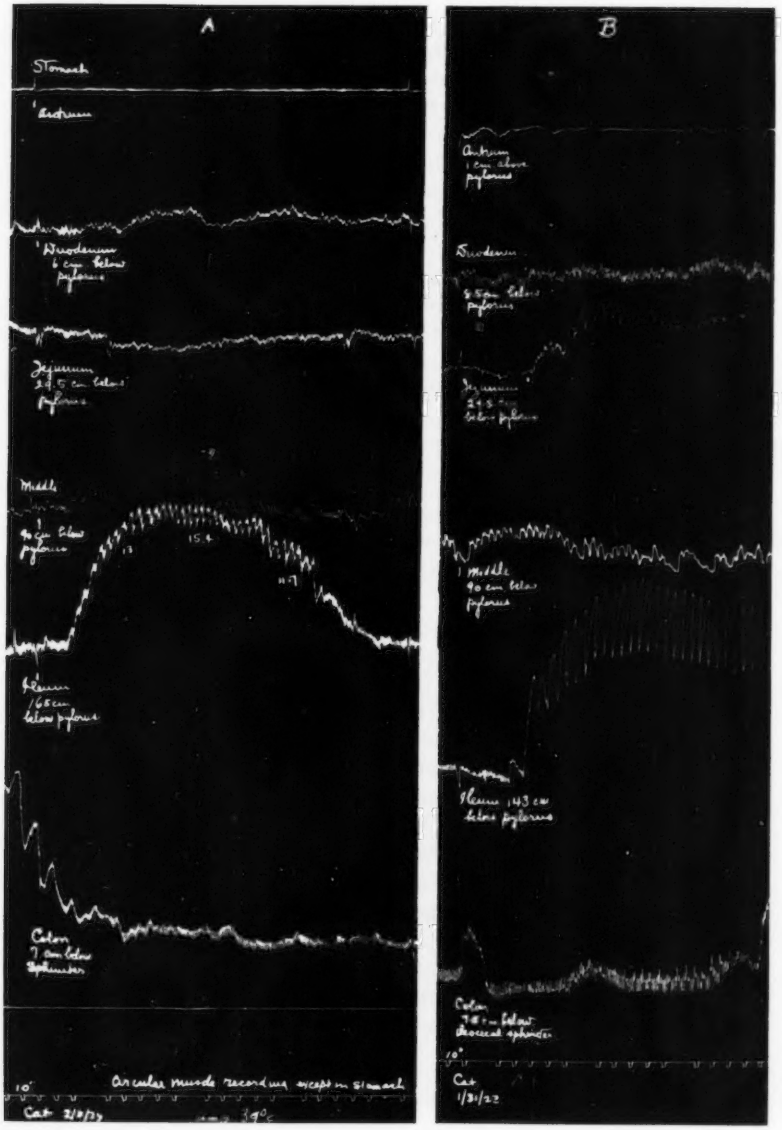

Fig. 3. Records from the stomachs and bowels of cats opened under salt solution.

whereas on the other side of the pyloric line, the muscle is much more active and contracts (in the rabbit) from 15 to 23 times per minute. These differences are marked even in small bits of excised muscle, and there is no question that there are certain optimal types and rates of contractions. What I find now, however, as I study more carefully the intact animal, is that the muscle has considerable versatility, and especially under unusual conditions, it can respond in a number of unusual ways. Thus, as I shall show in a subsequent paper, under certain conditions, as in intestinal obstruction, the pyloric antrum of a rabbit can contract rhythmically from 8 to 22 times a minute.

Those who are interested in the problem of the neurogenic or myogenic origin of the intestinal contractions will now be faced with the question: What agency is responsible for the tonus rhythm? Another question is: Are the tonus and the segmenting contractions due to changes in the physico-chemical state of two different substances in the muscle? These are problems for the future.

SUMMARY

In addition to the segmenting movements and the peristaltic rushes we find in the bowel slow tonus waves.

It is shown that in the duodenum and jejunum of the cat this tonus rhythm can exist alone.

This tonus rhythm is sometimes captured by the stomach and made to follow it for a time.

The tonus waves do not seem to travel along the bowel.

They appear in records from the excised bowel and in records made with a balloon in the jejunum of a man.

At the height of the tonus contraction there is sometimes a marked increase in the rate of the superimposed rhythmic contractions. Mere distention of the bowel does not ordinarily produce such an increase in rate.

Tonus waves in one part of the digestive tract will sometimes seem to affect positively or negatively the activities of another part.

These waves probably have a good deal to do with starting and stopping the peristaltic rushes.

Although the muscle in different parts of the digestive tract is specialized and suited to the work which must be done in those parts, it can, especially under unusual conditions, contract in a number of ways and with different rhythms.

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STUDIES OF EMOTIONAL REACTIONS

IV. Metabolic Rate[1]

CARNEY LANDIS

From the University of Minnesota

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That emotional disturbance does affect the metabolic rate seems to be a fact upon which all investigators of metabolism agree. Benedict (1921), DuBois (1924) and Jones (1921) as well as others, caution the clinician who wishes to use the basal metabolic rate (B.M.R.) for diagnostic purposes to avoid emotional disturbance of the patient.

An approach to the problem has been made through the study of B.M.R. in the insane and in psychoneurotic cases. Raphael and Parsons (1922) made the first study of the sort appearing in the literature. This was not a calorimetric study but an investigation of the amount of blood sugar rise in dementia praecox and manic-depressive cases, following the injection of 1 mgm. of epinephrin. They found that the curves representing the amount of blood sugar present in the blood stream following such an injection were slightly different. The small number of cases and the failure to give probable errors of the results leave the difference questionable. The authors, however, do not stress the point but merely say that it may be significant. Bowman and Grabfield (1923) have given a report of the B.M.R. of 50 cases of various types studied at the Boston Psychopathic Hospital. Of these only four had a B.M.R. above the normal ± 10 per cent. Two of these four were manic-depressive (depressive phase), one manic-depressive (mixed) and one psychopathy without psychosis. Forty-two cases gave minus readings, 27 of these being less than -10 per cent. Gibbs and Lemcke (1923) made a study of metabolism in 15 cases of manic-depressive insanity, 11 cases of dementia praecox and five of mixed psychoses. They found that definitely abnormal rates occur in psychotic patients in the more acute phases of their psychosis. Variations from normal were greater in dementia praecox than in manic-depressive insanity. The patients did not show sufficient evidence to explain the findings in terms of thyroid or pituitary disorders. They conclude that in functional studies of the psychoses the

[1] Number III of this series appeared in the Journal of Comparative Psychology, 1925, v, 220.

findings should be considered in relation to the symptoms and phases or stages presented by each patient as well as the clinical group to which the patient belongs. They give complete tables of their findings which include estimates of the degree of emotional disturbance of each patient at the time of the metabolic determination. Levine (1923) examined 100 psychoneurotic cases, each of which had one or more goiter signs. He found 15 cases in this group, each having a B.M.R. of over +10 per cent and four who were below −10 per cent. This is about one-half of the usual expectancy of the cases in the Great Lakes region where this study was made. He concluded that the high rates in psychotic cases were not, in the most cases, due to hyperthyroidism or exophthalmic goiter superimposed on a neurosis but rather that the thyroid disturbance is the direct consequence of the psychosis. Cummings (1923) reports that 41 out of 58 patients (78 per cent) suffering from a "fatigue" neurosis showed a B.M.R. of below −10 per cent. "In general, if the skin and hair were dry and the memory deficient one could be certain that the rate would be subnormal." He accounts for this by assuming that the central nervous system is not consuming oxygen at the usual rate, due probably to superadrenal insufficiency. Walker (1924) examined 44 patients suffering from dementia praecox (mostly stuporous cases) making some 200 determinations of metabolic rate on the group. He reports that 50 per cent of the cases showed a subnormal B.M.R., the average being −20 per cent. During remission the metabolic rate of these patients approached normal. In psychoses other than dementia praecox he found no deviation from the normal B.M.R. The injection of $\frac{1}{30}$ grain of strychnine hydrochloride relieved the stupor in several cases and with the passage of the stupor the B.M.R. approached normal.

Zeigler and Levine (1925), in a recent study of the influence of emotional states on metabolism, have more nearly approached the problem than any previous workers. They made tests on 14 psychoneurotic war veterans, taking first a normal B.M.R., then suggesting that the patient think of some unpleasant war experience during a second determination, and following this with a third or normal determination. Two individuals showed a decreased rate during the suggested emotion, while the other 12 showed a more or less marked rise. The notes taken during these determinations, showing a decreased rate, indicate no difference of reactions from those during cases of accelerated B.M.R. They considered it doubtful that the patient was completely relaxed during the suggested emotion. They conclude: 1, that psychoneurotics without goiter usually respond, when thinking about emotion-producing incidents, with an increased B.M.R.; 2, that the most frequent objective responses to emotion-producing stimuli were small changes in skin color, slight changes in rate and amplitude of respiration, and very fine tremors; 3, that some

patients showed an increase in B.M.R., with practically no objective reactions, and were not aware of any emotion whatever; 4, that lying apparently still in bed is not to be taken as a criterion of rest.

Grafe and Trauman (1920) suggested heavy work or depression (suggestion of inoperable cancer, brain tumor, etc.) to two normal subjects during deep hypnosis. As controls they used the hypnotic sleep of the same individuals with no suggestion, and in the case of one of the subjects, one determination of B.M.R. during normal sleep. The determinations made on the two subjects during suggested depression gave contradictory results of doubtful significance. They did show that the suggestion of heavy work failed to raise metabolism. In a subsequent study Grafe and Mayer (1923) using hypnotic suggestion of either depression or gaiety on 10 different subjects were able to show an average increase 7.6 per cent during hypnotic anxiety as compared to metabolism during hypnotic sleep. (These percentages are comparative throughout and of a different value from those based on the DuBois scale.) In the highest case the comparative increase was 25.2 per cent. In the two cases of suggested joy the increase was 4.3 and 3.9 per cent respectively, as compared to hypnotic sleep. The authors suggest that this increase in metabolism is due to an increase in the metabolism of the brain. They quote, in support of their hypothesis, the work of Alexander and Révész (1912) who showed that the intermittent flashing of a light into the eye of a dog raised the dog's metabolism (oxygen consumption) 7.2 per cent.

In the opinion of the writer, this work of Grafe and his associates is highly suggestive but far from conclusive. It suffers most of all from lack of normal controls. That is, he should have had extensive records of the normal B.M.R. of his subjects so that one would know to what extent the raise of metabolism during hypnotic suggestion really was differing from the normal range of metabolism for that subject. The fact that the first study (Grafe and Trauman, 1920), where several determinations were made on the same subject, gave contradictory and inconclusive results, shows that one cannot consider the results of the second study (Grafe and Mayer, 1923) conclusive. The results of the second study were based on two determinations on each subject; one during hypnotic suggestion, and one during hypnotic sleep. Furthermore, in several instances an interval of a week to a month occurred between these determinations. Why the authors did not use ordinary experimental control is difficult to understand.

Aub (1920) showed that traumatic shock in the cat was followed by a depressed metabolic rate, the decrease being proportional to the degree of the shock. He found that this change in metabolic rate was not due to the volume or the exertion involved in respiration, or in entirety to the fall in blood pressure. Crile (1922) gives a table of data indicating a

fall of metabolic rate (R.Q. falls from 0.832 to 0.745) in rabbits which had been badly frightened by dogs.

Aub (1922) has given a review of the literature concerning the relationship between the internal secretions and metabolism. His evidence shows that in all probability the action of the thyroid gland is not part of the mechanism of emotion, since the effect of thyroxin is slow in appearance and continues over a long period of time. That epinephrin increases metabolism rapidly and always in the same fashion, has been shown by Sandiford (1920), while Evans and Ogawa (1914) have shown that the action of epinephrin is direct upon the muscle as evidenced by its action on isolated heart muscle. Aub (1922) has shown in his own investigations that the high metabolic rate in muscles, taken from thyrotoxic animals, persisted when the nerves supplying the muscle were cut. In brief, there is evidence that hormone activity in the absence of nervous control will cause an increase in metabolism. Crile and Rowland (1922) found that emotional disturbance raised the brain temperature in rabbits. From this it could be argued that the activity of the central nervous system raised the metabolic rate. The experiment of Alexander and Révész (1912) also bears on the point. Finally, it goes without saying that muscular activity raises the metabolic rate.

The work to date may be summarized somewhat as follows:

1. Metabolic rate is not diagnostic or symptomatic of any particular psychosis or of the emotional content of that psychosis.

2. The phase of manic-depressive insanity, with its supposedly altered emotional content, may or may not affect the metabolic rate.

3. Unpleasant emotional disturbance following suggestion is usually attended by a rise in the metabolic rate.

4. Extreme emotional disturbance or traumatic shock in animals is attended by a decreased metabolic rate.

5. The suggested causes for metabolic increase during emotional disturbance are: *a*, increase of superadrenal or thyroid (?) activity; *b*, greater metabolism of the central nervous system; and *c*, increased muscular tonus or incomplete relaxation.

PURPOSE. This study takes as its purpose an attempt to answer, or shed light upon the following questions: Will slight emotional stimulation, such as is afforded by music, affect metabolism? What is the effect of pronounced emotional upset upon metabolism? Is metabolism a measure of the degree of emotional upset? What is the relationship between metabolic changes during emotion and other physiological reactions occurring at the same time?

METHOD. All metabolic determinations reported in this study were made by means of the Krogh (1923) apparatus, using a mouthpiece and nose clip. This is a closed circuit system in which the determination is

based upon the rate at which oxygen is consumed, CO_2 being absorbed out of the system by soda-lime. All computations were based on the Boothby and Sandiford (1921) nomographic charts, a respiratory quotient of 0.82 being assumed. All work was carried on under the standard conditions for determinations of the basal metabolic rate, viz.: prone on a cot with complete muscular relaxation for at least 20 minutes, no food for at least 12 hours previous to the determination (unless otherwise noted hereafter), records were taken of pulse and body temperature to assure a normal condition, and all determinations were made in a quiet room of a comfortable temperature.

Two experimental series were carried on. In the first or "music" series the routine was as follows: The subject reported at 8:30 a.m. without having had breakfast. After a 20-minute rest period, two basal determinations, each of 10 minutes duration, were made. Then a third determination was made during which four records of popular jazz music were played on a phonograph. Following this, two more basal determinations were made. Finally a determination was made during which the subject was instructed to clench his hands and tense his forearms but to endeavor to keep the rest of his body relaxed. Six men and four women, graduate students in psychology at the University of Minnesota, served as subjects in this series. All were in a normal healthy condition. Two of the men had had previous experience with metabolic determinations, all other subjects were unacquainted with the procedure.

The second or "upset" series was carried on as follows. Basal metabolic rate was determined every day for a 10- to 14-day period. Then a 2-day control period was introduced, during which the subject fasted 44 to 47 hours and went without sleep for 36 to 38 hours. During this period frequent B.M.R. determinations were made, together with graphic records of blood pressure (Erlanger method), thoracic and abdominal respiration; and during one metabolic determination gastric and rectal balloons were introduced, making possible the recording of stomach and rectal contractions. After the fast had continued for 44 to 47 hours, the subject was allowed to drink a quart of egg-nog of a known composition. The metabolic rate was then followed 6 or 7 hours longer, following which the subject was allowed to go home and to bed. An interim period of 5 to 6 days then intervened during which normal B.M.R. determinations were made every morning. Then the experimental period was introduced. The conduct of this period was the same as that of the control period, except that late in the afternoon of the second day the subject was given strong faradic stimulation through the palm of either hand. Metabolism was taken before, during and after this period of stimulation. Following this, a quart of egg-nog was again given the subject and the course of metabolism followed 6 to 7 hours after which the subject was

allowed to go home and to bed. B.M.R. determinations were made every morning for several days subsequent to this, and until there was no doubt that the metabolism had returned to normal.

Dr. K. S. Lashley (Ly.), Mr. L. E. Wiley (Wy.), and the writer (Ls.) served as subjects in this experiment. All were in good health and in normal physical condition. The routine outlined above was followed by Ly and Ls. Subject Wy. went through the series in a reversed order, that is, the experimental period preceded the control period, the latter lasting only one day. In the data which follows the records of Wy. are inverted so as to follow the order of Ly. and Ls. Since the records show the same general trends as those of Ly. and Ls. this procedure seems to be justified.

In the "music" series blood pressure and respiration records were taken simultaneously with the metabolic record; and in the "upset" series the physiological reactions noted above were taken simultaneously with metabolism. Other than this additional instrumentation, the determinations were made under standard conditions. With the exception of the records taken while the gastric balloon was being used, there is no evidence indicating that the presence of any of the other apparatus affected the metabolism in any way.

DATA. As has been stated above, all metabolic rates given in this study are expressed in terms of plus or minus percentages on the DuBois scale for normal individuals. The computations are made by use of the Boothby and Sandiford (1921) nomographic charts, a respiratory quotient of 0.82 being assumed.

Table 1 gives a summary of the results of the "music" series. It will be noted that with the exception of M.S. (range 14 per cent), the range of the normal determinations is well within the limits of the range for well trained subjects. All subjects agreed that the determination made during the music was much less irksome than any of the other determinations. Hence it might be said that there was a "feeling" of relief or pleasantness accompanying this situation which failed, however, to affect the metabolic rate. The instructions to the subjects to hold the hands clenched and the forearms tense during this last determination had a markedly variable effect. There was an average increase of ± 7.3 per cent. This increase should be a direct measure of the amount of muscular work done.

In table 2 is given an abstract of the results of the "upset" series. The age, height, weight and surface area of each subject are given from which anyone interested may easily trace back from the percentages to the calories per square meter per hour, etc., by the use of the nomographic charts. Figure 1 gives a graphic representation of the course of the metabolic rate throughout the period of this study. The abscissa

TABLE 1

Statement of the metabolic rate in terms of percentage on the DuBois scale for normal determinations and determinations made during music and when the subject was tensing his hands and forearms

SUBJECT	SEX	NORMAL		MUSIC		TENSE	
		Average metabolic rate	Range	Metabolic rate	Differs from normal	Metabolic rate	Differs from normal
C. L.	M.	−10	−9 to −12	−11	−1	−1	+9
C. J.	M.	−11	−7 to −14	−5	+6	+16	+27
A. T.	F.	−7	−4 to −9	−3	+4	+21	+28
L. W.	M.	−6	−4 to −10	−3	+3	+24	+30
T. L.	M.	−10	−7 to −11	−15	−5	−9	+1
M. S.	F.	+10	+3 to +17	+5	−5	+27	+17
J. B.	F.	−3	−1 to −5	−3	0	−4	−1
J. B.	M.	−7	−6 to −9	−7	0	+8	+15
R. H.	F.	−10	−6 to −12	−11	−1	−7	+3
H. E.	M.	−7	−5 to −9	−7	0	0	+7
Average			5 5		+0.1		−13.6
Probable error					2.1		7.3

TABLE 2

Statement of the metabolic rate in terms of percentage on the DuBois scale, for three subjects giving normal B.M.R.'s together with changes brought about in the metabolic rate by emotional disturbance, food, etc.

ITEM		SUBJECT		
		Ly.	Ls.	Wy.
1	Age	35	28	26
2	Height in inches	72	70	68
3	Weight in pounds	124	128	129
4	Surface area in square meters	1.70	1.72	1.69
5	Average normal B.M.R.	−12%	−6%	−3%
6	Number of normal B.M.R.s	26	29	29
7	Range of normal B M.R.	27%	11%	17%
8	P. E. of normal B.M.R.	±4.7%	±2.6%	±3.2%
9	Average B.M.R. during control fast and insomnia period, (omitting rates during gastric balloon)	−12%	−2%	−6%
10	B.M.R. during use of gastric balloon, control period	−2%	+6%	+8%
11	Average B.M.R. during experimental period, fast and insomnia, previous to preparation for electrical stimulation	−15%	−6%	−3%
12	B.M.R. during anticipatory period	+4%	+23%	+45%
13	B.M.R. during electrical stimulation	+2%	−4%	+31%
14	Average of metabolic rates after food, control period	+18%	+15%	
15	Average of metabolic rates after food, experimental period	+10%	+11%	+29%

scale is a variable one, as is indicated. In order that the curves might be set upon the same base line and hence afford an easier comparison, the average normal B.M.R. of each subject is considered as zero. If one wishes to express the actual percentages of the subjects, 12 per cent, 6 per cent and 3 per cent should be subtracted from each point on the graph for subjects Ly., Ls. and Wy. respectively. As has been stated, subject Wy. went through the experiment in a reverse order of procedure to that of Ly. and Ls. His record has been broken, transposed and graphed in the same order as that of the other two subjects.

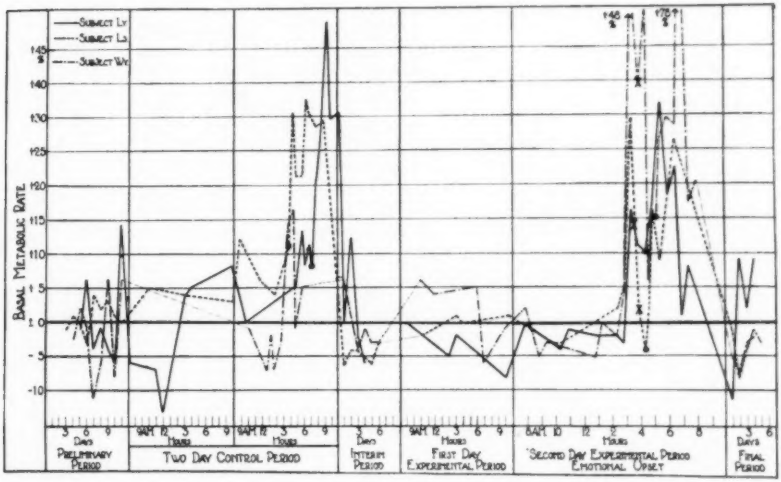

Fig. 1. Graphic representation of the course of the metabolic rate during the total period of study.

X—Metabolic rate during electrical stimulation.

O—Last metabolic determination before taking food.

(Note: The ordinate scale in this figure is incorrect, + 35 being omitted. It should read from the top down, +40 +35, +30, +25, etc.)

In table 3 is given a detailed account of the metabolic rate during the afternoon of the second day of both the control and the experimental periods. The time during which each determination is made is given so that the temporal relationship of the changes may be studied.

Results. The results of the "music" series indicate that jazz music does not offer sufficient stimulation to alter the metabolic rate. In a previous study (Landis and Gullette, 1925) the writer has shown that music will cause a moderate decrease in systolic blood pressure. Gilliland and Moore (1924) found that jazz music led to relaxation and slouch-

TABLE 3

Detailed statement of the metabolic rate during the afternoon of the second day of both the control and the experimental periods

COMMENT	SUBJECT Ly.		SUBJECT Ls.		SUBJECT Wy.	
	Metabolic rate in per cent	Time	Metabolic rate in per cent	Time	Metabolic rate in per cent	Time
Average basal metabolic rate	−12		−6		−3	
Control period:		March 21, '25. p.m.		March 5, '25. p.m.		April 5, '25. p.m.
After lavage and before balloon	−7	4:00 to 4:10			−4	1:30 to 1:40
					−10	1:50 to 2:10
During gastric balloon	+1	4:30 to 4:45	+6	8:15 to 8:30	−5	2:20 to 2:30
	−4	4:45 to 4:55			+11	2:40 to 2:50
After gastric balloon	−1	5:30 to 5:40		March 6, '25. p.m.	+13	3:00 to 3:10
	−4	5:40 to 5:50			−4	3:10 to 3:20
After first pint of egg-nog	+8	6:15 to 6:25	+24	4:10 to 4:20		
	+13	6:25 to 6:35	+15	4:30 to 4:40		
After second pint of egg-nog	+20	7:30 to 7:40	+18	5:30 to 5:40		
	+31	7:40 to 7:50	+12	6:15 to 6:25		
One hour after second pint of egg-nog	+17	9:00 to 9:10	+15	7:15 to 7:25		
	+18	9:10 to 9:20	+16	8:00 to 8:10		

Experimental period:		March 28, '25. p.m.		March 14, '25. p.m.		March 29, '25. p.m.
After lavage and before balloon	−14	3:30 to 3:40	−4	3:45 to 3:55	−5	3:40 to 3:50
	−15	3:40 to 3:50	−1	4:00 to 4:10	+1	3:50 to 4:00
Anticipatory period (during gastric balloon)	+4	4:10 to 4:16	+23	4:15 to 4:20	+45	4:25 to 4:32
During electrical stimulation	+2	4:16 to 4:31	−4	4:20 to 4:30	+31	4:32 to 4:39
After electrical stimulation	−1	4:23 to 4:33	−10	4:35 to 4:50	+42	4:39 to 4:45
	−2	4:35 to 4:45	−4	5:00 to 5:10	+3	4:50 to 5:00
					+12	5:10 to 5:20
					+12	5:20 to 5:30
After one pint of egg-nog	+5	5:30 to 5:40	+10	5:30 to 5:40	+23	6:10 to 6:20
	+19	5:40 to 5:50	+3	5:45 to 5:55	+26	6:20 to 6:40
After second pint of egg-nog	+6	7:10 to 7:20	+20	7:10 to 7:20	+25	7:30 to 7:40
	+10	7:20 to 7:30			+67	7:40 to 7:48
One hour after second pint of egg-nog	−11	8:20 to 8:30			+14	8:30 to 8:40
One hour after second pint of egg-nog	−3	8:30 to 8:40			+17	8:40 to 8:50
Two hours after second pint of egg-nog	−23	9:30 to 9:40				

ing as compared to classical music. Evidently the relaxation and drop of blood pressure are not of sufficient degree to be reflected in the metabolic rate.

The first point to be emphasized in the results of the "upset" series is the determination of the normal B.M.R. and its probable error (P. E.). For subject Ly. this was -12 per cent $\pm$ 5 per cent; for subject Ls. -6 per cent $\pm$ 3 per cent; and for subject Wy. $-$ 3 per cent $\pm$ 3 per cent. In terms of calories per square meter per hour these figures are 35.4 $\pm$ 1.2, 37.1 $\pm$ 1.0, and 38.2 $\pm$ 1.1 in order as above.

Neither the control nor the experimental period of fast (46 hours) and insomnia (34 hours) failed to change the B.M.R. to a figure exceeding the probable error for any subject. The metabolic determination made during the control period in which the gastric balloon was used shows an increase of 10 per cent (Ly.), 12 per cent (Ls.), and 11 per cent (Wy.). Not all of this rise can be attributed to the emotional disturbance accompanying the passage of the stomach tube and balloon, since it was necessary for the subject to sit erect on the edge of the cot in order to swallow the tube, so introducing some muscular activity. That the entire rise is not due to the exercise is evidenced by the fact that even after the subject had been lying down for twenty minutes with the balloon in place there was only a slight decrease in the metabolic rate.

The routine for the experimental period included an enema and gastric expression and lavage before the gastric and rectal balloons were used. (The metabolic records taken during or after this procedure are not included in the computations of item 11 of table 2.) After the gastric and rectal balloons were put in place during the experimental series, an "anticipatory" period of 5 to 8 minutes elapsed while physiological records, including metabolism, were taken; following this electrical stimulation was employed. The metabolic rates were 4 per cent (Ly.), 23 per cent (Ls.) and 45 per cent (Wy.) during this time. If we subtract out the effect of the gastric balloon and muscular activity as determined in the control period (item 10, table 2) we find that the effect of this anticipatory period was to increase the metabolism 6, 17 and 37 per cent, in order as above. Following this, faradic stimulation was applied through the palm of each hand, using dampened sponges as electrodes. The strength of this stimulation had been previously determined so that it was quite painful but still not so intense as to cause the subject to struggle. In the case of subject Ly. the first stimulation, due to a mistake, was very strong, so that he did struggle momentarily until the inductorium was readjusted. The subjects were instructed to take the stimulation as long as they could stand it, and when they felt that they were nearing the limit of their endurance they signalled for the stimulation to be discontinued. This period was about 6 minutes in each case.

During this stimulation the metabolic rates were +2 per cent (Ly.), −4 per cent (Ls.), and +31 per cent (Wy.). (Due to the fact that the breathing of the subjects during electrical stimulation was somewhat irregular, the fit of the line to the respiratory curve, from which the metabolic rate was figured, was rather difficult. As a compromise the average between the highest and lowest possible interpretations was used as the rate given above.) In each case there is a drop in rate from that of the anticipatory period. For Ly. this is only 2 per cent but, as has been said, the stimulation with this subject was such as to cause more muscular activity than for the other subjects. If we turn to table 1, we find that the subject L. W. (Wy.) showed a metabolic rate of +30 per cent and C. L. (Ls.) +9 per cent, during tensing of the hands and forearms as compared to the +31 per cent and −4 per cent during electrical stimulation. From this it would seem possible that the increase in metabolism during electrical stimulation might be due to the muscular activity of the hands and forearms.

The work of Hartree and Hill (1922) has shown that following muscular activity there is a delayed heat production of approximately 1.5 times the total initial heat production, which extends over some 20 minutes. In other papers Hill has termed this the "oxygen debt" of muscular activity. This being the case it seems reasonable to suppose that following the electrical stimulation with its consequent muscular tension and activity, we should expect to find an increase in metabolism. For Ly. and Ls. there is a marked decrease. Wy. shows an increase of 11 per cent immediately following stimulation. This subject choked at the end of electrical stimulation and in the removal of the gastric balloon he moved about considerably. Hence it is possible that his metabolism would otherwise have fallen as did that of Ly. and Ls. Indeed his metabolism did fall very pronouncedly within 11 minutes after the end of electrical stimulation, as reference to table 3 will show. This action of the metabolic rate, falling during electrical stimulation and continuing to fall after the stimulation ceases, seems inexplicable in terms of any of the three hypotheses so far advanced to explain the metabolic changes attendant on emotional disturbance.

A reflection of the emotional disturbance due to the electrical stimulation is seen in the comparative effect of the food (egg-nog) taken at the end of the control and of the experimental period (items 14 and 15, table 2). This stimulation was not sufficient to totally stop stomach contractions, as was shown by the gastric balloon records; but the utilization of the food was slowed down 8 per cent and 4 per cent in subjects Ly. and Ls. From figure 1 it will be seen that the course of metabolism following the experimental period does not differ significantly from the course during the interim period. This indicates that the electrical

stimulation, although it brought about an undoubted emotional upset, did not affect the organism in other than a transient fashion; metabolic rate is not a direct measure of emotional disturbance or "upset."

During the period of several weeks which this upset period covered, it happened that on several occasions the subject became emotionally disturbed at the time during which a metabolic determination was being made. These findings were as follows:

OCCASION	VERBAL REPORT	METABOLIC RATE	DIFFERS FROM NORMAL B.M.R.
		per cent	*per cent*
Subject Ly.:			
Apparatus difficulties	"Angry"	−18	−6
Apparatus difficulties	"Angry"	+51	+63
Overheard conversation	"Inhibition of laughter"	+76	+88
Subject Ls.:			
Apparatus difficulties	"Angry"	−12	−6
Apparatus difficulties	"Angry"	+6	+12
Subject Wy.:			
Not enough sleep	"Peevish"	−9	−6
Not enough sleep	"Peevish"	−1	+2
Very hungry	"Peevish"	−10	−7

This indicates that the emotion of "anger" does not always have the same effect, each subject showing rates of contradictory direction.

On the record blank for each metabolic determination a space was provided for "subject's attitude." Other than the entries tabulated just above, no correspondence or facts worthy of note were obtained. This may be due to the fact that the attitude of the subject was usually the same, early morning sleepiness and impatience with the routine which must be gone through before breakfast.

The results of the other physiological records, and the discussion of the general behavior of the subjects during the experiment, will be taken up in a subsequent paper.

Conclusions. The fact that the records of the three subjects employed in the "upset" series are comparable throughout, showing no contradictions, and always changes in the same direction with the same change of conditions, demonstrates that the phenomena exhibited here are not due to chance but that we are dealing with some common factor. However, the results of this experiment are not easy to interpret, the results being indicative rather than conclusive. That it is possible to cause changes in the metabolic rate (rate of oxygen consumption) experimentally in response to emotion-arousing stimuli is clear. The physiological basis for these changes is a matter of conjecture. The quick rise

during the anticipatory period followed by a rapid fall during electrical stimulation, is a more rapid change than those due to the intravenous injection of epinephrin (Sandiford, 1920). That the natural secretion of epinephrin should lead to a different effect from that of intravenous injection is scarcely to be expected.

Increased muscular tonus (incomplete relaxation) does account for part of the rise, but the evidence does not indicate that the entire rise may be accounted for on this basis. If anything, tonus should be higher during electrical stimulation than during the anticipatory period, the metabolic rate would increase instead of fall. It is possible, of course, that the subject lay in a very tense condition during the anticipatory period, and that during the electrical stimulation his body relaxed other than his hands and arms, since his attention was directed entirely to the pain in his hands. However, there was nothing in the observable behavior of the subject, or in the verbal reports which the subject gave following the experiment, which in any way favors this hypothesis. In brief there does not seem to be sufficient evidence to indicate that muscular tonus or incomplete relaxation accounts for more than a small portion of the metabolic rise in the experiment.

The explanation suggested by Grafe (1923), viz., that the metabolic rate of the central nervous system is markedly increased during emotional disturbance, remains. The evidence here is meager. Grafe was able to show an increase as high as 25 per cent (from −2 per cent to +17 per cent on the DuBois scale, if our interpretation of his figures is correct) over the control. This was during hypnotic suggestion and he states that the relaxation was complete and unquestionable. Crile and Rowland (1922) report than when a rabbit was badly frightened by a dog, the temperature of the brain rose from 35.8°C. to 37.1°C. in 1 minute, approximated this figure for 7 minutes and then fell to 35.2°C. during the following 5 minutes. A temperature change of this magnitude in the human brain would presumably involve oxidative processes to an extent which would account for some portion of the metabolic changes in emotional disturbance. However, the body temperature of a rabbit is extremely variable and the evidence gained from the study of this animal may be questioned on the basis of the variability of the material used. Similarly the rise of 7.2 per cent in the metabolism of the dog, as reported by Alexander and Révész (1912), is well within the limits of the normal probable error of the metabolic rate of the dog.

The fall of blood pressure, behavior of the subjects, and the decreased rate of metabolism suggest that the mechanism involved in this emotional upset which we have caused is of a similar nature to that of mild surgical shock. Aub (1920) states that the explanation of the decreased rate of metabolism in shock is obscure. He suggests that the work of Gesell

(1918) may be to the point. Gesell found that in the early stages of shock from tissue abuse there was usually a marked reduction of the volume-flow of blood in peripheral organs although the blood pressure was but slightly changed. The volume-flow determines the oxygen delivery to the tissues and hence the rate of oxygen consumption (metabolic rate). From this it may be tentatively suggested that the variations of metabolic rate in this experiment were due in some part to muscular activity; but more largely to changes in the volume-flow of blood, attendant on vascular changes giving a redistribution of the blood.

Other types of emotional stimulation may lead to other varieties of emotional reactions, with consequent differences in the metabolic changes. In such instances the variation may be caused by incomplete relaxation, by the secretion of epinephrin or even thyroxin. The point being that emotional disturbance *per se*, does not lead to changes in the metabolic rate which are always in the same direction or of the same magnitude. Conversely, changes in metabolic rate cannot be considered as direct measures of immediate emotional disturbance or of cumulative emotional upset. The interrelationship between the various physiological reactions and metabolism will be taken up in a later paper.

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SUMMARY

1. The literature concerning the metabolic rate of psychotic patients is reviewed with reference to the relationship between the emotional states of the patients and their metabolism. It is shown that altered or changed emotional conditions do not necessarily affect the metabolic rate.

3. A 46-hour fast, coupled with 34 hours of insomnia, failed to affect the metabolic rate.

4. Anticipation of strong electrical stimulation raised the metabolic rate 6 per cent, 17 per cent and 37 per cent (DuBois scale) over the

normal basal rate of three subjects. Electrical stimulation together with the presence of a gastric balloon caused an increase of 14 per cent, 2 per cent and 34 per cent over the basal rate of these same subjects.

5. The emotional disturbance caused by the electrical stimulation, although it failed to stop stomach contractions, slowed down the metabolic rate after the taking of food 8 per cent and 4 per cent in two subjects.

6. "Anger" is sometimes accompanied by an increase of metabolism and other times by a decrease.

7. It is suggested that the variations in metabolic rate exhibited in this experiment are partly due to muscular activity but for the most part to vascular changes involving the volume-flow of the blood.

7. Emotional disturbance, *per se*, does not lead to changes in the metabolic rate which are always in the same direction or of the same magnitude. Conversely, changes in metabolic rate cannot be considered as direct measures of emotional disturbance or cumulative emotional upset.

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HEAT REGULATION AND WATER EXCHANGE

X. Water, Salt and Lipoid Accumulation in the Serum as a Preliminary to Sweating[1]

H. G. BARBOUR, M. H. DAWSON and I. NEUWIRTH

From the Department of Physiology and Pharmacology and the Department of Pathology, School of Medicine, University of Louisville

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Until the significance of blood dilution and concentration in response to heat and cold respectively was pointed out by one of us (Barbour, 1921), the evidence indicating these changes had consisted chiefly in altered red cell counts, while attention had always been centered upon cells rather than fluid. Perhaps the chief reason for the lack of appreciation of the water factor lay in the fact that most observations upon the response to heat were not carried out under such conditions as distinguished clearly between the phase of blood dilution and the later one of anhydremia due to sweating. For example, Barbour and Tolstoi (1924), even in attacking the problem from the new point of view, were not wholly successful in separating these two phases owing evidently to variations in environmental humidity and air motion.

Control of hot room conditions with the kata-thermometer has afforded the means of clear-cut demonstration of the phase of blood dilution. Barcroft and his collaborators (1922) have shown that when the appropriate conditions are long continued, as in acclimatization to tropical climates, the hydremia becomes compensated by the acquisition, first, of stored, and later, of new red blood cells.

Bazett (1924) reports observations on the phase of blood dilution in men taking warm baths. The hydremia has been missed by some investigators because the proper atmospheric conditions were never attained. For dogs, Lozinsky (1924) succeeded in establishing the proper temperatures both in moist and dry environments.

At a "wet-kata" cooling-power of about 8 millicalories per square centimeter per second, Barbour, Loomis, Frankman and Warner (1924) observed in man a fall in hemoglobin sometimes attaining 8 or 10 per cent while the blood solids showed constantly a drop of a few

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tenths of 1 per cent. Hamilton, Barbour and Loomis (1924) showed that the specific gravity of the blood both in dogs and man exhibited variations similar to the total solids but that as long as the environmental conditions were not severe, a tendency to an increase of the $\frac{\text{specific gravity}}{\text{solids}}$ ratio was seen. This was interpreted as indicating a relative increase in the blood salts which of course are the heaviest constituents per volume.

In the present investigations we wished, first, to establish beyond all doubt the constancy of blood dilution in man under exposure to heat and the fact that it is not a matter of cell loss but of fluid increase. We also undertook to learn something of the nature of the serum changes, including evidences of exchange between the blood cells and plasma, and especially, to study the significance of the whole complex for the sweating process.

PROCEDURES AND METHODS. The experiments were all carried out on fourteen medical students in the third decade of life and in apparently good health. Details of height, weight and age are given in table 5. They were exposed to warm environments by use of the hot chamber previously described (Barbour, Loomis, Frankman and Warner, 1924); the dry bulb temperature varied between 30.9° and 38.5°C., and the wet kata-thermometer was depended upon as the best indicator of the physiological stimulus. Conditions were controlled in each case by varying either the ventilation or the humidity of the room, the latter being regulated by a jet of steam. One observer usually remained in the chamber with the subject and the kata-thermometer was read just before rather than after the visits of others for blood samples, which were made as brief as possible.

The experiments were divided into two series according to the severity of the conditions. The environment in the first series (with eight subjects) is referred to as "moderately warm," in the second series (with the other six subjects) as "hot." The wet-kata cooling-power of the "moderately warm" experiments was maintained in the neighborhood of from 6 to 9 millicalories during the first half or three-quarters of an hour after which it was reduced to a lower level. In the "hot" experiments the cooling-power varied from about 5 to 1 millicalories. The subjects entered the chamber early in the afternoon (at about 2:00 o'clock), having fasted since the previous day except for a light breakfast. They were clad only in athletic underwear and footgear and were first kept at absolute rest in the main room at a temperature of about 20°C. Samples of about 12 cc. of blood were withdrawn from a vein of the forearm just before entering the chamber and at intervals of about one-half hour thereafter, as shown in the tables. The general condition of each subject was followed by reference to the pulse rate, oral temperature and occurrence and severity of sweating (table 5).

In the whole blood *hemoglobin* was determined colorimetrically by the

method of Cohen and Smith, using the Newcomer glass as a standard, *specific gravity* by the falling drop method (Barbour and Hamilton, 1924).

The *total solids* were found by drying the blood to a constant weight in an oven between 100 to 110°C. About 8 cc. of each blood sample was allowed to clot and then centrifuged. Serum *specific gravity* and *total solids* were determined as for whole blood; the *protein* content was calculated from nitrogen determinations made by digestion and aeration and the *ash* content by combustion in porcelain crucibles in a furnace at dull red heat. From the unidentified fraction of the serum solids the probable value of the *lipoid* content was calculated. All the above determinations were made in duplicate.

The serum changes reported in tables 2 and 4 were calculated as follows:

G = specific gravity minus 1.0

S = total solids (grams in 100 grams serum)

P = protein grams in 100 grams serum = $\frac{6.25\,(N-0.026)}{\text{specific gravity}}$ (0.026 is deducted for non-protein N)

A = salt grams in 100 grams serum = ash determination $\times$ 1.053 (estimated loss of CO_2 from total solids)

L = calculated lipoids = $S - (A + P + 0.16)$

The figures designated as L are considered to represent the lipoid content of the serum on the supposition that this is the only significant unaccounted-for fraction of the solids after protein and ash have been deducted and a further allowance made of -0.16 per cent (by weight), -0.1 per cent for dextrose and 0.06 per cent for non-protein nitrogen constituents. The correctness of this supposition has been verified in this laboratory by direct determination of the ether soluble fraction in various types of sera.

$\frac{\text{Specific gravity}}{\text{solids}}$ ratio: As first published, the falling drop method is well adapted to the determination of relative changes in the fourth decimal place of specific gravity. As used in the present work, it, therefore, affords a reliable means of studying *changes* in the $\frac{\text{specific gravity}}{\text{solids}}$ ratio of blood and serum. However, owing to the limitations of the Westphal balance, absolute values can not be relied upon beyond the third place. A modified method, giving highly accurate absolute values is, however, now nearly ready for use. For these reasons the $\frac{\text{specific gravity}}{\text{solids}}$ ratio has not, for the present purposes, been calculated numerically; but in the subjoined figures (1 to 4) its fluctuations can readily be followed either in blood or serum by studying the convergence or divergence of the respective curves of specific gravity and total solids. The first observations in each experiment are superimposed, and 20 per cent solids corresponds well enough to a specific gravity of 1.050 to justify the evaluation of each one per cent change in solids as equal to a change of 0.0025 in specific gravity.

TABLE 1

Whole blood changes in moderately warm environments

(Initial Wet-katas from 11.6 to 7.0 millicalories)

SUBJECT	TIME	WET-KATA	HEMOGLOBIN		SPECIFIC GRAVITY		SOLIDS	
			Per cent	Difference from normal		Difference from normal	Per cent	Difference from normal
	minutes	*millicalories*						
D. D.	0	11.6	93		1.0578		21.19	
	30	9.4	88.5	−4.5	1.0566	−0.0012	21.24	+0.05
	120	8.9	85	−8.0	1.0564	−0.0014	20.6	−0.59
M. W.	0		95		1.0609		22.03	
	28	8.2	92	−3.0	1.0591	−0.0018	21.85	−0.18
	65	7.3	98	+3.0	1.0596	−0.0013	21.85	−0.18
	96	6.9	96	+1.0	1.0592	−0.0017	22.05	+0.02
H. K.	0	8.2	79		1.0528		19.13	
	10*	6.2*	73	−6.0*	1.0530	+0.0002*	19.03	−0.10*
	30	7.6	77	−2.0	1.0518	−0.0010	19.17	+0.04
	65	7.3	77	−2.0	1.0515	−0.0013	19.2	+0.07
S. H.	0	9.8	83.5		1.0566		20.19	
	45	7.3	79	−4.5	1.0562	−0.0004	20.30	+0.11
	80	5.9	75	−8.5	1.0552	−0.0014	20.07	−0.12
	130	3.5	74	−9.5	1.0564	−0.0002	20.45	+0.26
G. W.	0	9.8	74		1.0544		20.46	
	30	7.3	72.5	−1.5	1.0543	−0.0001	20.03	−0.43
	75	5.9	73	−1.0	1.0546	+0.0002	20.44	−0.02
	110	3.5	72	−2.0	1.0545	+0.0001	20.21	−0.25
R. S.	0	7.5			1.0555		21	
	34	7.25			1.0581	+0.0026	21	0
	77	1.87			1.0576	+0.0021	20.75	−0.25
W. S., Jr.	0	7.6			1.0516		20.9	
	30	7.2			1.0511	−0.0005	20.9	0
	60	1.5			1.0537	+0.0021	21	+0.1
C. N.	0	7			1.0499		21.18	
	30	7.33			1.0499	0	21.06	−0.12
	60	1.54			1.0519	+0.0020	21.29	+0.11
Average change	28–45	9.4–7.2		−3.8		−0.0003		−0.066
	60–80	7.3–1.5		−2.1		+0.00034		−0.041
	96–130	8.9–3.5		−4.6		−0.0008		−0.140

* This 10 minute sample not included in averages.

GENERAL EFFECTS. The effects on pulse rate, body temperature and sweating detailed in table 5 may be summarized as follows:

The *pulse rate* changes varied in the first series from 0 to + 22 beats per minute and in the second group (exposed to more severe conditions) from +10 to +25 per minute.

The *body temperature* changes in the first series varied from −0.3° to +0.6° except for one obese subject in whom the temperature increased by 2.45°C.

In the second series the changes varied from +0.45° to +1.55°C.

Sweating: Careful records were made of the time when sweating first appeared at various locations and it was not regarded as "severe" until such large areas as the chest, abdomen or back were involved. The onset was irregular in the first group of experiments; in one case it did not occur at all and in three of the eight it was delayed for over an hour. Sweating became severe in only half of the cases in this group and never within the first hour. In the six experiments of the second group sweating began promptly and became severe in from 40 to 75 minutes.

BLOOD CHANGES IN MODERATELY WARM ENVIRONMENTS. The whole blood changes induced by "moderately warm" environments appear in table 1. Definite reductions are seen in the hemoglobin percentage in each of five cases examined, the maximum change being −9.5 per cent. In one case (M. W.) there was a fall of 3 per cent in the first half hour but the net change from normal at the end of the first hour was +3 per cent. In no other instance was the hemoglobin increased above normal. The decrease in the specific gravity and solids of the whole blood were much less pronounced than in the hemoglobin, as in the experiments reported by Hamilton, Barbour and Loomis (1924). The solids, however, showed a definite diminution in six of the eight cases. Likewise there is a significant fall in the specific gravity in five of the eight cases. The very much greater change in the hemoglobin indicates the acquisition by the blood of fluid approaching in concentration and specific gravity the original blood.

The $\frac{\text{specific gravity}}{\text{solids}}$ ratio is of particular interest (fig. 1). At first a fall in the ratio is indicated by the lesser decline of the solids curve; at the end of the second period the ratio increased above normal. This latter increase resembles that noted by Hamilton, Barbour and Loomis (1924),

Fig. 1. Whole blood changes in "moderately warm environments." (Averages from table 1.) Circles: hemoglobin; crosses: solids; dots: specific gravity.

Zero ordinate is normal.

Fig. 2. Serum changes in "moderately warm environments." (Averages from table 2.) Triangles: lipoids (calculated); oblongs: ash; diamonds: protein; crosses: solids; dots: specific gravity.

Zero ordinates: upper is normal for lipoids, ash and protein, lower is normal for solids and specific gravity.

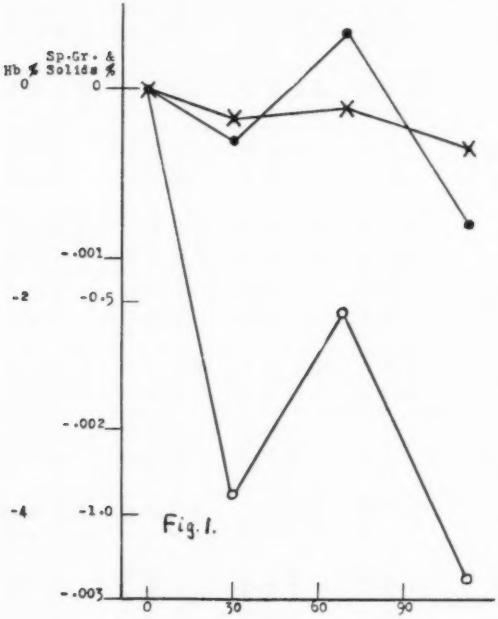

Hb %
Sp.Gr. &
Solids %
0
0
-.001
-2
-0.5
-.002
-4
-1.0
-.003
0
30
60
90

Fig. 1.

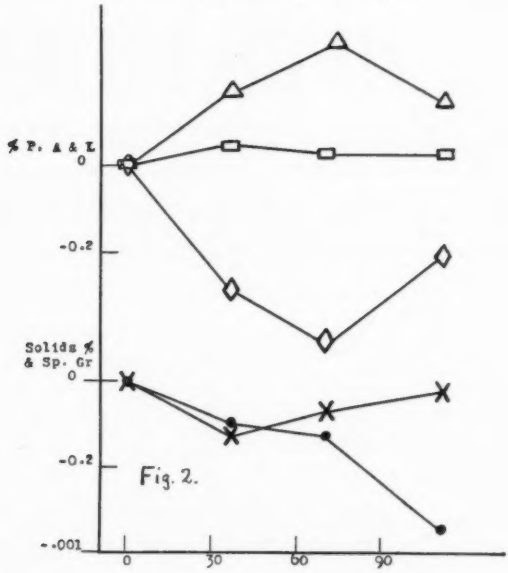

% P. A & L
0
-0.2
Solids %
& Sp. Gr
0
-0.2
-.001
0
30
60
90

Fig. 2.

TABLE 2

Serum changes in moderately warm environments

(Initial wet-katas from 11.6 to 7.0 millicalories)

SUBJECT	TIME	WET-KATA	G		S		P		A		L	
				Difference from normal	Per cent	Difference from normal	Per cent	Difference from normal	Per cent	Difference from normal	Calculated per cent	Difference from normal
	minutes	*millicalories*										
D. D.	0	11.6	0.0265		9.40		6.36?		0.79		2.09?	
	30	9.4	0.0264	−0.0001	9.49	+0.09	6.28?	−0.08?	0.87	+0.08	2.18?	+0.09?
	120	8.9	0.0269	+0.0004	9.40	0	6.27?	−0.09?	0.85	+0.06	2.12?	+0.03?
M. W.	0		0.0246		8.88		7.29		0.80		0.63	
	28	8.2	0.0244	−0.0002	8.77	−0.11	7.17	−0.12	0.83	+0.03	0.61	−0.02
	65	7.3	0.0245	−0.0001	8.71	−0.17	6.88	−0.41	0.81	+0.01	0.86	+0.23
	96	6.9	0.0239	−0.0007	8.86	−0.02	7.23	−0.06	0.82	+0.02	0.65	+0.02
H. K.	0	8.2	0.0238		8.63		6.84		0.78		0.85	
	10*	6.2*	0.0238	0*	8.35	−0.28*	6.15	−0.69*	0.79	+0.01*	1.25	+0.40*
	30	7.6	0.0234	−0.0004	8.38	−0.25	6.25	−0.59	0.79	+0.01	1.18	+0.33
	65	7.3	0.0232	−0.0006	8.68	+0.05	6.63	−0.21	0.84	+0.06	1.05	+0.20
S. H.	0	9.8	0.0262		9.30		7.27		0.83		1.04	
	45	7.3	0.0255	−0.0007	9.03	−0.27	6.76	−0.51	0.89	+0.06	1.22	+0.18
	80	5.9	0.0244	−0.0018	9.10	−0.20	6.57	−0.70	0.87	+0.04	1.50	+0.46
	130	3.5	0.0242	−0.0020	9.24	−0.06	7.11	−0.16	0.85	+0.02	1.12	+0.08
G. W.	0	9.8	0.0253		8.78		6.67		0.84		1.12	
	30	7.3	0.0251	−0.0002	8.78	0	6.56	−0.11	0.90	+0.06	1.16	+0.04
	75	5.9	0.0247	−0.0006	8.78	0	6.34	−0.33	0.86	+0.02	1.42	+0.30
	110	3.5	0.0242	−0.0011	8.77	−0.01	6.15	−0.52	0.87	+0.03	1.59	+0.47

W. S., Jr.	0	7.6	0.0238		9.24							
	30	7.2	0.0237	−0.0001	8.99	−0.25						
	60	7.5	0.0243	+0.0005	9.06	−0.18						
C. N.	0	7.0	0.0239		9.25							
	30	7.33	0.0239	0	9.10	−0.15						
	60	1.54	0.0245	+0.0006	9.31	+0.06						
Average change	28–45	9.4–7.2		−0.00024		−0.124		−0.282		+0.048		+0.18
	60–80	7.5–1.54		−0.00033		−0.073		−0.412		+0.032		+0.297
	96–130	8.9–3.5		−0.00085		−0.023		−0.207		+0.032		+0.15

* This 10 minute sample not included in averages.

which was taken to indicate a mobilization of salt. This hypothesis has been amply confirmed by the direct evidence from the ash changes in the serum, to be described below. Yet it must be admitted that the $\frac{\text{specific gravity}}{\text{solids}}$ increase is not seen in the whole blood or even in the serum with the same constancy as the augmentation in its ash content. For another factor enters into the interpretation of specific gravity changes in blood and serum, namely, the content in lipoids.

In the serum the "moderately warm" environment diminished both the specific gravity and solids (table 2) in all experiments except the first (the least severe exposure). This appears to be the first demonstration that the serum as well as the whole blood dilutes in response to hot environments. Barbour and Hamilton's (1925a) converse finding in dogs exposed to cold baths will be discussed below. As will be seen from figure 2, the $\frac{\text{specific gravity}}{\text{solids}}$ ratio was but little altered at the end of the first period, the slight increase, however, in the presence of a decided augmentation in the lipoid content indicating a mobilization of salt into the serum. This has been established by the ash determinations.

It is of particular interest to compare the $\frac{\text{specific gravity}}{\text{solids}}$ ratio of the serum with that of the whole blood which shows at the time of the first hot room sample a decrease rather than an increase. In terms of salt alone this would mean some loss of salt from the blood as a whole but entirely at the expense of the cells, accumulation taking place in the serum. The later decline in the specific gravity of the serum shown in the figure is associated with the accumulation of lipoids.

The protein percentage decrease (table 2 and fig. 2) brings out strikingly the inflow of fluid into the serum.

The lipoid changes as estimated in the manner described above are all consistently in the direction of increase, this amounting to nearly one-half per cent of the serum in three different subjects. The curve of average change for five cases (fig. 2) emphasizes the importance of this feature.

A striking feature of the blood curves in the "warm environment" series (fig. 1) is the extensive increase in the specific gravity of the whole blood, paralleled by a similar rise in the hemoglobin, seen in the third samples (taken 60 to 80 minutes after entrance into the warm room). Such changes were found in five of the seven subjects from which samples were taken at this stage, and were marked enough to affect the average curves very significantly. There is no reflection of these changes in the serum curves. If due simply to a large amount of plasma leaving the blood, one would expect a like increase in the total solids of the whole blood.

In the absence of evidence sufficient to establish definitely the origin of these delayed hemoglobin and specific gravity increases, a tentative sug-

gestion may be made which seems to fit the known facts. Barcroft (1922) and his collaborators have shown that the relative dilution of the blood in hot environments tends sooner or later to be compensated by the acquisition of red blood cells presumably from the splenic reservoir. That such augmentation may occur somewhat spasmodically is seen in the report of their two hot chamber experiments and accords with Barcroft's theory of

TABLE 3

Whole blood changes in hot environments

(Initial wet-katas from 4.1 to 3.9 millicalories)

SUBJECT	TIME	WET-KATA	HEMOGLOBIN		SPECIFIC GRAVITY		SOLIDS	
			Per cent	Difference from normal		Difference from normal	Per cent	Difference from normal
	minutes	*millicalories*						
W. M.	0		89.0		1.0540		20.3	
	24	4.3	86.5	−2.5	1.0537	−0.0003	20.2	−0.1
	83	2.6	83.0	−6.0	1.0527	−0.0013	19.78	−0.52
	133	1.7	80.5	−8.5	1.0533	−0.0007	20.27	−0.03
D. G.	0	3.9	79.5		1.0524		19.69	
	32	3.3	75.0	−4.5	1.0520	−0.0004	19.28	−0.41
	95	2.9	78.5	−1.0	1.0517	−0.0007	19.52	−0.17
L. B.	0	4.1	89.0		1.0571		21.27	
	40	4.9	85.0	−4.0	1.0559	−0.0012	21.19	−0.08
	90	1.3	86.5	−2.5	1.0562	−0.0009	20.86	−0.41
W. C.	0	3.9	91.5		1.0563		20.88	
	36	3.7	90.0	−1.5	1.0550	−0.0013	20.75	−0.13
	62	1.2	92.0	+0.5	1.0547	−0.0016	20.48	−0.40
L. N.	0		82.0		1.0531		19.24	
	24	3.7	79.5	−2.5	1.0521	−0.0010	19.19	−0.05
	53	2.1	82.0	0	1.0520	−0.0011	19.43	+0.19
Average change	24–40	4.9–3.3		−3.0		−0.00084		−0.154
	53–83	2.6–1.2		−1.98		−0.00133		−0.243
	90–133	2.9–1.3		−4.0		−0.00076		−0.203

the emergency function of the spleen. It is conceivable that the results referred to represent in five of our subjects a flooding of the circulation with cells from the splenic reservoir and that these cells are of a different composition from those already present in the circulation. We have pointed out above that one of the first reactions to heat is the giving up of salt by the cells of the serum. The new cells acquired from the splenic reservoir

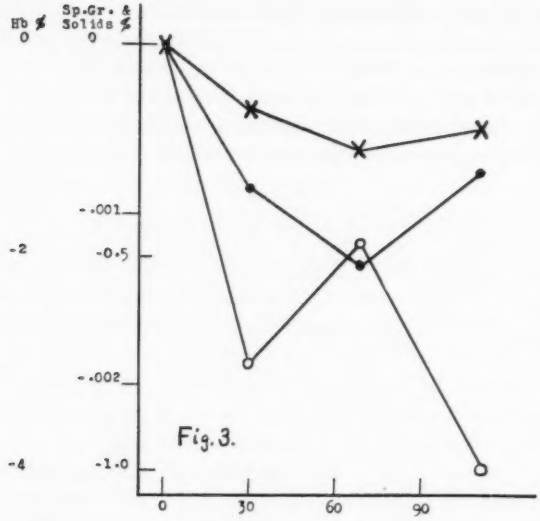


Fig. 3.

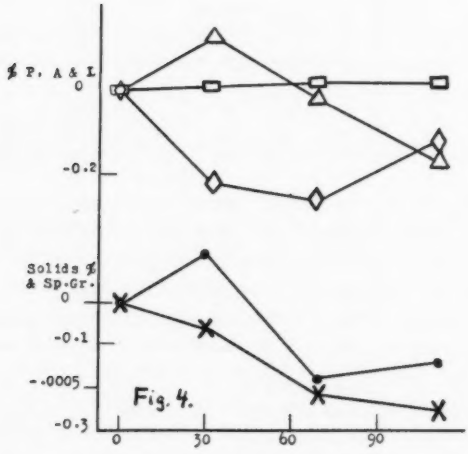


Fig. 4.

Fig. 3. Whole blood changes in "hot environments." (Averages from table 3.) Key same as for figure 1.

Fig. 4. Serum changes in "hot environments." (Averages from table 4.) Key same as for figure 2.

would probably be richer in salt and poorer in lipoids than those already in the blood. This would help to compensate for processes which have already taken place, and would account not only for the great increase in

the $\frac{\text{specific gravity}}{\text{solids}}$ ratio at this time, but also for the succeeding decline in lipoids and continued mobilization of salt into the serum.

BLOOD CHANGES IN HOT ENVIRONMENTS. The blood changes in environments in which the facilities for giving off heat were further reduced are summarized in tables 3 and 4 which relate to whole blood and serum respectively. The wet-kata cooling-power was adjusted to about four millicalories at the beginning of each experiment and decreased to one or two at the end.

In spite of the fact that these conditions invariably caused sweating to begin very early and to become very profuse, this loss of fluid from the circulation continued to be more than compensated for throughout each experiment. The phase of dilution may, therefore, persist for two hours or longer under rather severe conditions.

The average hemoglobin curve (fig. 3) is similar to that obtained under the more moderate conditions but none of the changes are quite as extreme. The specific gravity and total solids of the whole blood were, however, particularly affected by the heat, the average gain of fluid being much greater in this series than in the first. Attention may now be called to the fact that there is agreement in all fourteen experiments as to the occurrence of dilution of the whole blood by whatever method determined.

The $\frac{\text{specific gravity}}{\text{solids}}$ ratio unlike that in the first series shows a marked and persistent decrease in accordance with the loss of salt from the blood cells with sweating.

The serum changes in the more severe environments differed in several respects from those noted in the first series. The fall in total solids was more marked, the salt increase less so, the latter fact conforming probably to the draft made upon the blood for salt by the sweat glands.

Special attention is called to the uniformity of the individual experiments (table 4) with respect to the changes in lipoid content. In each of four experiments in which the lipoids were calculated accumulation was seen during the first half-hour, followed by subsequent loss. This loss, not seen in our first series, was associated with the occurrence of heavy sweating, when lipoids are known to accumulate outside the skin.

The $\frac{\text{specific gravity}}{\text{solids}}$ ratio of the serum in the hot environments was maintained throughout at a higher level than normal (fig. 4). This may be accounted for by the increase in the salt content and must be contrasted with the reversed condition in the whole blood, again showing the draft on the cells for salt.

DISCUSSION. The fundamental fact brought out by this series of experiments is that exposure to environments with low cooling-power causes an accumulation of fluid in the blood. This is proven by the decline in specific gravity, solids and hemoglobin of the whole blood as well as in the

TABLE 4

Serum changes in hot environments

(Initial wet-katas from 4.1 to 3.9 millicalories)

SUBJECT	TIME	WET-KATA	G		S		P		A		L	
				Difference from normal	Per cent	Difference from normal	Per cent	Difference from normal	Per cent	Difference from normal	Calculated per cent	Difference from normal
	minutes	*millicalories*										
A. B.	0		0.0237		8.96		6.78		0.81		1.21	
	28	4.3	0.0239	+0.0002	8.95	−0.01	6.72	−0.06	0.83	+0.02	1.24	+0.03
	85	2.6	0.0222	−0.0015	8.54	−0.42	6.44	−0.34	0.82	+0.01	1.21	0
	134	1.7	0.0227	−0.0010	8.75	−0.21	6.68	−0.10	0.81	0	1.09	−0.12
W. M.	0		0.0226		8.46		6.53		0.80		0.98	
	24	4.3	0.0217	−0.0009	8.34	−0.12	6.21	−0.32	0.77	−0.03	1.20	+0.22
	83	2.6	0.0218	−0.0008	8.20	−0.26	6.34	−0.19	0.75	−0.05	0.94	−0.04
	133	1.7	0.0223	−0.0003	8.28	−0.18	6.54	+0.01	0.80	0	0.79	−0.19
D. G.	0	3.9	0.0225		8.92		7.24		0.83		0.69	
	32	3.3	0.0222	−0.0003	8.84	−0.08	7.00	−0.24	0.89	+0.06	0.79	+0.10
	95	2.9	0.0219	−0.0006	8.78	−0.14	7.12	−0.12	0.90	+0.07	0.60	−0.09
L. B.	0	4.1	0.0216		9.51		7.81		0.87		0.67	
	40	4.9	0.0231	+0.0015	9.37	−0.14	7.55	−0.26	0.86	−0.01	0.80	+0.13
	90	1.3	0.0221	+0.0005	9.04	−0.47	7.56	−0.25	0.88	+0.01	0.44	−0.23
W. C.	0	3.9	lost		lost							
	36	3.7	0.0257		8.13							
	62	1.2	0.0262		8.48							

L. N.	0		0 0256		9 11							
	24	3.7	0.0265	+0.0009	9 18	+0.07						
	53	2.1	0 0265	+0 0009	9.14	+0.03						
Average change	24–40	4.9–3 3		+0 00028		−0 056		−0.220		+0.010		+0 120
	53–85	2.6–1 2		−0.00046		−0 216		−0.265		+0 020		−0 020
	90–134	2.9–1 3		−0 00036		−0 250		−0.115		+0.020		−0.157

TABLE 5

Subjects, atmospheric conditions and general effects

SUBJECT	WEIGHT	HEIGHT	AGE	DRY BULB RANGE	WET BULB RANGE	WET-KATA RANGE	PULSE RATE		BODY TEMPERATURE		SWEATING	
							Range per minute	Increase per minute	Range	Increase	Began after	Severe after
	lbs.			*°C.*	*°C.*	*millicalories*			*°C.*	*°C.*	*minutes*	*minutes*
D. D.	175	5′ 10½″	26	31.6–33.0	23.3–25.2	11.6–8.9	72–72	0	36.4–37.0	0.6	<10	
M. W.	140	5′ 6″	25	32.0–33.7	26.0–27.0	8.2–6.9	66–78	12	36.9–37.2	0.3	60	100
H. K.	155	6′	25	32.0–33.7	26.0–27.2	8.2–7.3	56–78	22	37.1–36.8	−0.3	65	
S. H.	167	5′ 5¾″	23	30.9–36.9	22.4–31.5	9.8–3.5	62–68	6	37.0–39.45	2.45	<10	80
G. W.	165	5′ 11″	22	33.2–36.9	27.0–31.5	9.8–3.5	61–74	13	37.0–37.35	0.35	60	75
R. S.	136	5′ 10″	27	35.4–34.1	23.4–29.7	7.5–1.87	70–84	14	36.8–37.15	0.35		
W. S. Jr.	148	5′ 6″	20	33.5–34.3	23.0–31.4	7.6–1.5	62–82	20	37.2–36.95	−0.25	20	
C. N.	170	5′ 10″	26	32.5–34.0	23.0–31.4	7.0–1.54	74–88	14	37.0–37.6	0.6	<10	60
A. B.	126	5′ 5½″	22	33.3–35.7	27.8–32.5	4.3–1.7	80–94	14	36.4–37.3	0.9	<10	40
W. M.	130	5′ 7½″	23	32.8–36.1	27.8–32.7	4.3–1.7	74–98	24	36.65–37.1	0.45	<10	70
D. C.	135	5′ 6½″	22	36.1–38.4	27.1–30.6	3.9–2.9	71–96	25	36.4–37.35	0.95	20	40
L. B.	150	6′ 2″	23	36.1–38.5	27.1–32.6	4.1–1.3	74–84	10	36.4–37.35	0.95	20	50
W. C.	185	5′ 10¼″	29	34.4–38.2	28.0–37.0	3.9–1.2	64–80	16	36.5–37.1	0.6	<10	75
L. N.	144	5′ 7½″	25	34.4–38.4	27.0–35.0	3.7–2.1	58–80	22	36.0–37.55	1.55	<10	40

solids, specific gravity and protein of the serum. It is quite beyond the bounds of possibility that these changes represent merely a loss of the various solid constituents which one measures in making these determinations. Were the changes in the whole blood interpreted as simply an escape of cells from the circulation, one would still have to account for the dilution of the serum. It is not reasonable to suppose that the total volume of actively circulating blood would be diminished under the effect of moderate heating by such an elaborate process as loss of cells accompanied by escape of serum protein leaving a smaller volume of diluted blood to be further drawn upon by the sweating mechanism. When one sees the surface of body flushed and the peripheral blood flow greatly augmented and water being freely given off at the body surface, the logic of the situation demands a greater and not a lesser blood volume.

Direct proof that the total blood volume is increased seems to be furnished by the experimental results of the Barcroft expedition (1922). Their findings with the carbon monoxide method show that at least that part of the circulating blood which is not too narrowly confined to permit the movement of red blood cells is decidedly increased under the influence of heating. Furthermore, Barbour and Hamilton (1925 a) have recently demonstrated a concentration of the blood serum in dogs exposed to cold. Here in the converse of the hot room experiments something is moved about besides red blood cells; and the same authors (Hamilton and Barbour, 1925) have demonstrated that this movement consists in the taking up of fluid by skin, subcutaneous tissue and muscle.

Denial of all red blood cell movement in connection with fluid shifting in heat regulating processes would, however, be inconsistent with the present state of our knowledge, for the accumulation of such cells in the circulation has been shown by Barcroft and his collaborators to be a delayed response to heat by which the augmentated blood volume is made up to proper composition. The hemoglobin fluctuations seen in our experiments might, as suggested above, be interpreted in part as due to the cell shifting activity of the spleen which that author has so convincingly described. The significant thing, however, in the response of the blood to a warm environment is, as has been emphasized for several years by one of us, the mobilization of fluid. More heat can thus be carried to the surface of the body and more water made available for evaporation.

That salt should be mobilized with the water is what one would expect in connection with any physiological movement of fluid; in this case material for sweat is thus provided.

That there is a temporary mobilization of lipoids followed by a loss under the severer conditions accords with the undoubted fact of the increased oleosity of the surface of the body during sweating. It should be made clear that we do not at present feel justified in placing too much emphasis

on the quantitative value of the calculated L as an estimation of the lipoid content of the serum. It represents that part of the serum solids which can not be otherwise accounted for. In most instances the value of L found in the normal subject agreed well with expectation (e.g., the average value of serum lipoids for normal man given by Gram (1924) is 0.67 per cent by volume) but what requires emphasis is the consistency with which marked increases in the value of L have been found in every case under the mild conditions of heating, as well as slight increases followed by a decrease below normal in all the more severe experiments. At the present writing one of us is engaged jointly with Doctor Hamilton upon work in which this method of estimating lipoids is being subjected to severe scrutiny. In various types of sera are determined the total solids, protein and ash content and a constant allowance of 0.16 made for dextrose and non-protein nitrogen constituents. The ether soluble fraction of the serum is then compared with the calculated L. The agreement is usually found complete within about 0.1 per cent by weight of the total serum. A formula for the specific gravity components of each of the more important constituents of the serum is also being tested. This has been applied to all the cases in the present paper in which the value of L has been calculated, and the specific gravity of the L fraction in the majority of cases has been found to lie in the neighborhood of 1.0 which from what is known of their specific gravities when dry, would be expected of a mixture of cholesterol and fatty acids. By two different indirect methods of calculation therefore the mobilization of lipoids before sweating is indicated.

SUMMARY. Experiments upon the exposure of fourteen normal men to warm environments have been divided into two groups according to the severity of the conditions. Eight subjects were exposed to an initial wet-kata cooling-power of 11.6 to 7.0 millicalories; the other six to from 4.3 to 3.7 millicalories.

Conditions in the first series were such as to produce no severe sweating during the first hour but in four instances it became marked during the second. Except for one obese individual the maximum temperature increase in this group was 0.6°C.

In the more severe experiments the conditions were such that the onset of sweating was never delayed more than twenty minutes and it usually became severe in less than an hour. Here the temperature increases ranged from 0.6° to 1.55°C.

Dilution of the blood prevailed throughout both series of experiments as evidenced by half-hourly samples which showed diminution in hemoglobin, total solids and specific gravity of the whole blood as well as in total solids, specific gravity and proteins of the serum.

In the first group the hemoglobin and serum proteins suffered greater

reductions than in the second, while the reverse was true of the total solids of both whole blood and serum.

Just as the above findings indicate the mobilization of water for sweating, so the ash determinations reveal a similar mobilization of salt. The first group showed an average ash increase above the normal of about 0.04 per cent by weight, the second group, of about 0.02 per cent.

Besides the water and salt there was a marked accumulation of lipoids (calculated) in the blood in the first series, the average increase at the end of one hour amounting to about 0.3 per cent by weight. In the second group the lipoids increase was limited to the first hot room sample averaging only 0.12 per cent and falling at the end of the experiments to 0.16 per cent below normal. Thus while the increases in water and salt were partially maintained, the lipoids fell below normal with the onset of sweating.

Since in the first hot room samples in both series the $\frac{\text{specific gravity}}{\text{solids}}$ ratio of whole blood was found decreased while that of the serum was increased, this is taken as probable evidence of the accumulation of salts in the serum at the expense of the blood cells. This is a known accompaniment of decreased venosity (Van Slyke, Wu and McLean, 1922); and decreased venosity upon exposure to heat has been demonstrated by Meakins and Davies (1920), Goldschmidt and Light (1925) and others. The red blood cells might, therefore be considered the primary source of the sweat.

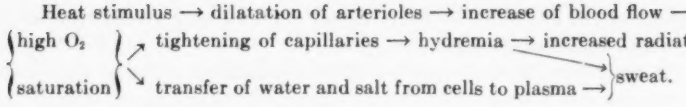
In some of the experiments the evidence suggests an inflow of salt-rich blood cells at the end of the first hour.

The results of the present investigations illustrate how greatly the respiratory mechanisms of the blood contribute toward the regulation of body temperature. Barbour and Hamilton (1925b) outlined this relation in the case of cold as follows:

(1) Direct cooling of arterioles, or
(2) Cooling of cutaneous nerves → axone spinal and prespinal reflexes } → constriction of arterioles → slowing of blood flow → capillary anoxemia → capillary relaxation → increased permeability → { edema / anhydremia

Edema, by insulation, affords some protection against cold. Anhydremia does so by limiting water loss and by lessening peripheral circulation, thus reducing radiation.

In the regulation against heat one sees that the sweat production is, in at least two ways, dependent upon the respiratory condition of the blood. The results described in the present paper suggest the following scheme:



CONCLUSIONS

1. The cardinal findings in this work on exposure to warm environments are the mobilization into the serum of water, salts and lipoids. The primary source of the first two appears to be the red blood cells.

2. The accumulation of water in serum as well as whole blood shows that the hydremia of adjustment to external heat is not relative but absolute, in accord with the complementary findings of loss of water from the serum as well as blood in dogs exposed to cold (Barbour and Hamilton).

3. The phase of blood dilution continues until sweating becomes severe enough to swing the balance toward anhydremia.

4. Rapid and accurate specific gravity determinations by the falling drop method are not only of much value in indicating the water content of blood or serum but, studied in conjunction with the total solids, afford a clue to changes in other constituents. In this way was first conjectured the mobilization of salt for sweating, confirmed herein by ash determinations. Specific gravity calculations have confirmed that the undetermined fraction L of the solids (augmented in warm environments) represents the lipoid elements.

5. In the more severe environments, which early produced abundant sweating, the serum salt percentage was found less increased than where the atmospheric cooling-power was higher. The salt level, however, remained elevated in both cases, affording a continuous supply for sweating.

6. The lipoids on the other hand appeared to cease mobilizing as sweating progressed. Presumably they contribute in some way to the sebaceous secretion. This of course does not evaporate, hence there is no continued demand.

7. The respiratory condition of the blood is significantly related to the regulation of body temperature. The response to hot environments is bound up with increased oxygen saturation of peripheral capillary and venous blood, a condition which favors both hydremia and sweat production.

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